Umbilical Cord Mesenchymal Stem Cell Transplantation in Severe and Refractory Systemic Lupus Erythematosus

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Objective. Umbilical cord (UC)–derived mesenchymal stem cells (MSCs) have shown marked therapeutic effects in a number of diseases in animal studies, based on their potential for self-renewal and differentiation. No data are available on the effectiveness of UC MSC transplantation (MSCT) in human autoimmune disease. This study was undertaken to assess the efficacy and safety of allogeneic UC MSCT in patients with severe and treatment-refractory systemic lupus erythematosus (SLE).

Methods. We conducted a single-arm trial that involved 16 SLE patients whose disease was refractory to standard treatment or who had life-threatening visceral involvement. All of the patients gave consent and underwent UC MSCT. Clinical changes were evaluated before and after transplantation using the SLE Disease Activity Index (SLEDAI), measurement of serum antinuclear antibody (ANA), anti–double-stranded DNA (anti-dsDNA) antibody, serum complement C3 and C4, and albumin levels, and assessment of and renal function. Evaluation of potential mechanisms of MSCT effects focused on the percentage of peripheral blood Treg cells and serum levels of cytokines.

Results. From April 2007 to July 2009, a total of 16 patients with active SLE were enrolled and underwent UC MSCT. The median followup time after MSCT was 8.25 months (range 3–28 months). Significant improvements in the SLEDAI score, levels of serum ANA, anti-dsDNA antibody, serum albumin, and complement C3, and renal function were observed. Clinical remission was accompanied by an increase in peripheral Treg cells and a re-established balance between Th1- and Th2-related cytokines. Significant reduction in disease activity was achieved in all patients, and there has been no recurrence to date and no treatment-related deaths.

Conclusion. Our findings indicate that UC MSCT results in amelioration of disease activity, serologic changes, and stabilization of proinflammatory cytokines. These data provide a foundation for conducting a randomized controlled trial of this new therapy for severe and treatment-refractory SLE.

Systemic lupus erythematosus (SLE) is an inflammatory disease with protean manifestations, ranging from relatively minor skin and joint symptoms to severe life-threatening major organ involvement, such as nephritis and neuropsychiatric complications (1). It is characterized by the presence of autoreactive T and B lymphocytes, with polyclonal activation of B cells and the consequent production of autoantibodies by plasma...
cells and release of cytokines (2). One of the most significant clinical features of SLE is lupus nephritis, which affects >50% of patients and is a major cause of morbidity and mortality (3).

Conventional immunosuppressive or immunomodulatory therapy, such as glucocorticoids, cyclophosphamide (CYC), and mycophenolate mofetil (MMF), can control disease in most, but not all, patients with lupus nephritis. There is a subset of lupus nephritis patients whose disease either does not respond or relapses despite continuing chemotherapy, and their prognosis remains poor. In addition, progressive immunosuppressive therapy may lead to the development of serious infection, cumulative drug toxicity, and an increased risk of cardiovascular disease and malignancy (4). In the last decade, hematopoietic stem cell transplantation (HSCT) has been reported as a promising therapy to achieve treatment-free, long-term remission in lupus (5), but the rates of relapse and treatment-related toxicity are high, as are the rates of the development of a secondary autoimmune disorder (6). Therefore, newer therapeutic approaches with enhanced efficacy and less toxicity than current treatment standards are urgently needed.

Mesenchymal stem cells (MSCs) are widely studied as an alternative cell source for their ability to differentiate into multiple mesenchymal lineages, including bone, fat, and cartilage (7). Recent studies have indicated that these pluripotent cells also differentiate into endoderm and neuroectoderm lineages, including neurons, hepatocytes, and cardiocytes (8–10). An important function of MSCs for autoimmune diseases is their immunomodulatory effect on various activated lymphoid cells, such as T cells, B cells, natural killer cells, and dendritic cells (11–13). MSCs express low levels of HLA class I major histocompatibility complex (MHC) molecules and are negative for class II MHC costimulatory molecules such as CD80, CD86, and CD40 (14). MSCs directly suppress activated T cell proliferation in an antigen-independent and dose-dependent manner. These characteristics support the possibility of using MSCs for therapeutic applications in autoimmune diseases.

Although MSCs were originally isolated from bone marrow (BM), similar populations have been isolated from other tissues, including adipose tissue (15), placenta (16), amniotic fluid (17), and fetal tissues, such as fetal lung and blood (18). In addition, the umbilical cord (UC) is a rich source of MSCs (19). Flow cytometric analysis revealed that CD29, CD44, CD95, and CD105 were expressed on the cell surface of UC MSCs, but there was no expression of hematopoietic lineage markers, such as CD34, CD38, CD71, and HLA-DR (20). In addition, colony-forming unit fibroblast frequency was higher in UC nucleated cells than in BM nucleated cells. UC MSCs had a higher proliferation capacity and lower levels of expression of CD106 (21), indicating that UC MSCs may be a novel alternative source of human MSCs for clinical application.

Recently, we found that BM MSCs from SLE patients were functionally abnormal, with alterations in the cytoskeleton and with increased autophagosomes and apoptotic bodies (Sun L, et al: unpublished observations). Of interest, MSCs from normal BM and UC were found to significantly ameliorate lupus nephritis and serologic changes in MRL/lpr mice with active nephritis (22). Furthermore, we found a pronounced therapeutic effect of BM MSCs in patients with treatment-refractory SLE (23). Therefore, in the present study, we sought to determine the therapeutic effect of UC MSCs in severe and treatment-refractory SLE.

PATIENTS AND METHODS

Patient selection. From April 2007 to July 2009, 16 SLE patients ranging in age from 17 to 56 years, were enrolled in an MSCT trial. All enrolled patients met at least 4 of the 11 American College of Rheumatology criteria for SLE (24). The eligibility criteria included progressive and active disease, with an SLE Disease Activity Index (SLEDAI) score of ≥8, lack of response to treatment with monthly intravenous pulse CYC (500–1,000 mg/m²) for ≥6 months or lack of response to treatment with oral MMF (2,000 mg/day) for ≥3 months, and continued daily doses of >20 mg of prednisone or its equivalent. Patients were also included if they had refractory immune-mediated transfusion-dependent thrombocytopenia or refractory lupus nephritis, regardless of whether they met the eligibility criteria described above. Refractory lupus nephritis was defined as either proteinuria ≥1,000 mg/24 hours, or serum creatinine ≥1.5 mg/dl, or decreased creatinine clearance without end-stage renal failure in patients with World Health Organization class IV/V glomerulonephritis despite 6 months of treatment with CYC or 3 months of treatment with MMF. Patients were excluded from this study if they had uncontrolled infection, mean pulmonary artery pressure >50 mm Hg, failure of one of the vital organs, or were pregnant or lactating. The trial was conducted in compliance with current Good Clinical Practice standards and in accordance with the principles set forth under the Declaration of Helsinki, 1989. This protocol was approved by the Ethics Committee at The Drum Tower Hospital of Nanjing University Medical School, and informed consent was obtained from each patient.

UC MSC purification and identification. UC MSCs were prepared by the Stem Cell Center of Jiangsu Province (Beike Bio-Technology). Fresh UCs were obtained from informed, healthy mothers in local maternity hospitals after normal deliveries and were processed as quickly as possible.
The cords were rinsed twice in phosphate buffered saline in penicillin and streptomycin, and the cord blood was removed during this process. The washed cords were cut into 1-mm² pieces and floated in low-glucose Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. The pieces of cord were subsequently incubated at 37°C in a humidified atmosphere consisting of 5% CO₂. 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 10 days, the cultures were trypsinized and passaged into a new flask for further expansion.

All of the infused UC MSCs were derived from passages 2–5, with rigorous purification and quality control. Cell viability of purified MSCs was >92% (as determined by trypan blue testing), and each preparation was negative for pathogenic microorganisms, including aerobic and anaerobic bacteria (as determined by direct cultivation analysis), and negative for hepatitis B surface antigen, hepatitis B core antibody, hepatitis C virus antibody, human immunodeficiency virus antibodies I and II, cytomegalovirus IgM, and syphilis antibody (as determined by enzyme-linked immunosorbent assay [ELISA]). Flow cytometric analysis showed CD29, CD73, CD90, and CD105 expression of 92% (as determined by amebocyte lysate analysis) in the supernatants of Tachypleus UC MSCT protocol. Before UC MSCT, 11 of the patients were each given a total of 0.8–1.8 gm of CYC because of their poor medical condition or myelosuppression. The other 5 patients received no CYC because of their poor medical condition or myelosuppression before transplantation. Cells (1 × 10⁶ per kg of body weight) were administered by intravenous infusion with no premedication such as steroids or antihistamines.

After UC MSCT, the dose of prednisone was tapered by 5–10 mg every 2 weeks during the first month following transplantation. All of the patients were given prednisone at 5–10 mg/day, and 13 patients also received CYC (0.6–0.8 gm per 2–3 months) as maintenance therapy. No other immunosuppressant was used unless the disease relapsed. A withdrawal schedule was followed to taper off prednisone and prolonged usage of CYC if the patient’s condition continued to improve. Patients were to be excluded from this protocol if relapse occurred, in which case the patient would receive other medications or a second course of MSCT.

**Followup procedures.** All 16 patients successfully completed the protocol. All patients were followed up at 1 and 3 months, 10 patients were followed up at 6 months, and 2 patients were followed up for >1 year. Assessments performed at these time points included physical examination, determination of SLEDAI score, serologic studies, and evaluation of organ functions if the results were previously abnormal. If a patient was not able to return for followup, medical records and laboratory measures were collected from their local physician or medical facility. Adverse events and their severity were assessed and recorded throughout the study.

**Laboratory methods.** Heparinized peripheral blood samples (2 ml) were collected, and mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Fluorescein isothiocyanate–conjugated anti-CD4, phycoerythrin (PE)–conjugated anti-FoxP3, and PE-conjugated anti-human IgG2a were all obtained from eBioscience. The percentage of peripheral blood CD4+FoxP3+ Treg cells was analyzed by flow cytometry according to the recommendations of the manufacturer, and flow cytometry data were analyzed with CellQuest software (Becton Dickinson). At least 5.0 × 10⁵ T cells were acquired. Serum samples were collected before

<table>
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<th>Patient/age/sex</th>
<th>Disease duration, months</th>
<th>SLEDAI score before MSCT</th>
<th>Length of followup, months</th>
<th>Clinical manifestations</th>
<th>WHO classification†</th>
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* SLE = systemic lupus erythematosus; SLEDAI = SLE Disease Activity Index; MSCT = mesenchymal stem cell transplantation.
† World Health Organization (WHO) classification of renal biopsy findings.
MSCT and at each followup visit. Antinuclear antibodies (ANA) and anti–double-stranded DNA (anti-dsDNA) antibodies were assessed by ELISA (OUMENG). Values for ANA were calculated as (optical density [OD]serum/ODcontrol). Concentrations of Th1-, Th2-, and Treg-related cytokines (interferon-γ [IFNγ], interleukin-4 [IL-4]), transforming growth factor β [TGFβ], and IL-10) were measured by ELISA (R&D Systems).

Statistical analysis. The paired t-test was used to compare data for each patient before and after transplantation using GraphPad Prism software, version 4.03. P values less than 0.05 (2-tailed) were considered significant.

RESULTS

Study sample. Sixteen patients with severe and treatment-refractory SLE (14 women and 2 men with a mean ± SD age of 31.8 ± 11.3 years [range 17–55 years]) were enrolled and underwent UC MSCT. Their mean disease duration was 65.1 ± 53.9 months (range 2–168 months), and their mean length of followup was 8.25 months (range 3–28 months). Ten patients completed ≥6 months of followup, and 2 patients were followed up for >2 years. Patient demographic and clinical characteristics are shown in Table 1. The treatment protocol for each patient before and after MSCT is presented in Table 2.

Assessment of disease activity. Mean ± SD SLEDAI scores for all 16 patients with severe and refractory SLE decreased significantly 1 month after UC MSCT (10.8 ± 0.8 at 1 month versus 18.4 ± 1.1 at baseline; n = 16) (P < 0.001) and further improved after 3 months (7.9 ± 0.8; n = 16) (P < 0.001 versus before MSCT). For the 10 patients who were followed up for ≥6 months, SLEDAI scores decreased gradually (7.3 ± 0.7 at 6 months versus 19.0 ± 1.6 before MSCT; n = 10) (P < 0.001). Two patients (patient 1 and patient 2) completed 2 years of followup, and their SLEDAI scores remained <4 (Figure 1).

Renal function. Twenty-four–hour proteinuria was measured at each visit in the patients who had lupus nephritis (n = 15), and serum creatinine and urea nitrogen levels were measured at each visit in the patients who had impaired renal function at baseline. There was reduced proteinuria in each of 15 patients at the 3-month visit (mean ± SD 1,338.0 ± 910.3 mg versus 3,121.5 ± 1,191.4 mg at baseline; n = 15) (P < 0.001).
Eight patients had a further decrease in proteinuria after 6 months (1,056.5 ± 866.5 mg versus 3,237.4 ± 1,259.9 mg at baseline; n = 8) (P = 0.001) (Figure 2A). The 2 patients who were followed up for >2 years both remained negative for proteinuria at 1 year and at 18 months (with evaluations repeated at least 2 times). Six patients had elevated serum creatinine levels at baseline (3.99 ± 2.19 mg/dl), and this measure improved significantly at 3 months (2.25 ± 1.06 mg/dl; n = 6) (P = 0.035 versus before MSCT) and at 6 months (1.82 ± 0.88 mg/dl; n = 3) (P = 0.026 versus before MSCT). In patient 2, serum creatinine levels decreased to normal at 1 year of followup and did not increase throughout the remainder of the followup period. Serum urea nitrogen levels also decreased in the patients who had elevated serum creatinine levels at baseline (Figure 2B).

**Changes in serologic features.** In the 13 patients with hypoproteinemia (mean ± SD 23.56 ± 1.53 gm/liter), serum albumin levels increased 3 months after UC MSCT (31.63 ± 0.96 gm/liter; n = 13) (P < 0.001 versus before MSCT) and reached almost normal levels after 6 months of followup (34.63 ± 1.75 gm/liter; n = 7) (P < 0.001 versus before MSCT) (Figure 3). In patient 13, who had protein-losing enteropathy, the serum albumin level reached 36.5 gm/liter 3 months after MSCT, compared with 18.1 gm/liter at baseline, in parallel with the amelioration of diarrhea. Serum C3 levels in 5 patients (0.44 ± 0.05 gm/liter at baseline) improved to 0.69 ± 0.04 gm/liter 3 months after MSCT (P = 0.05). In

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**Figure 1.** Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores before and after umbilical cord-derived mesenchymal stem cell transplantation (MSCT). Circles represent individual patients. Bars show the mean ± SD.

**Figure 2.** A, Levels of urinary protein in a 24-hour collection before and after mesenchymal stem cell transplantation (MSCT). Diamonds represent individual patients. Bars show the mean ± SD. B, Levels of serum creatinine and urea nitrogen at each visit in 6 patients.
patients 1 and 2, serum C3 levels reached 1.05 gm/liter and 0.97 gm/liter, respectively, at the 12-month visit and did not decline thereafter. Levels of anti-dsDNA antibodies decreased significantly 3 months after MSCT in 13 patients (316.6 ± 423.7 IU/ml at 3 months versus 414.4 ± 401.2 IU/ml at baseline; n = 13) (P = 0.003) and increased after 6 months (2.01 ± 1.59 ng/ml versus 0.92 ± 0.43 ng/ml; n = 3) (P = 0.07) (Figure 4B), while no significant changes were found in IL-10 concentrations (Figure 4C).

**Detection of Th1 and Th2 cytokines.** Previous studies have identified an imbalance of Th1 and Th2 cells in SLE patients. We analyzed the effect of MSCT on these 2 T cell subsets in the patients in the present study. Serum levels of IL-4 decreased substantially 3 months after MSCT (33.11 ± 2.33 pg/ml versus 37.36 ± 2.31 pg/ml at baseline; n = 12) (P = 0.02), in parallel with a decrease in Th1 cytokines (Figure 4C). Recent studies have identified several cytokines related to Treg cell proliferation and differentiation. The most relevant are considered to be TGFβ and IL-10. We measured the changes in the concentrations of these 2 cytokines and found that the concentration of TGFβ increased significantly 3 months after MSCT (3.06 ± 1.60 ng/ml versus 1.96 ± 0.98 ng/ml at baseline; n = 13) (P = 0.003) and increased after 6 months (2.01 ± 1.59 ng/ml versus 0.92 ± 0.43 ng/ml; n = 3) (P = 0.07) (Figure 4B), while no significant changes were found in IL-10 concentrations (Figure 4C).

**Systemic improvement.** In 6 of the patients with refractory cytopenias, the platelet count increased to 83 ± 56 × 10^9/liter 1 month after MSCT (versus 46 ± 32 × 10^9/liter at baseline) (P = 0.13) and further increased after 3 months (91 ± 7 × 10^9/liter versus 49 ± 11 × 10^9/liter at baseline; n = 5) (P = 0.01). Two of the 16 patients had central nervous system involvement (seizures in patients 2 and 7), and neither demonstrated recurrence after UC MSCT. Severe hypertension was controlled satisfactorily in the affected patients after UC MSCT and was accompanied by amelioration of renal function abnormalities.

**Changes in Treg cells and related cytokines after UC MSCT.** To detect the possible mechanism of the response to UC MSCT, we compared the percentage of CD4+FoxP3+ T cells (Treg cells) in peripheral blood before and after MSCT and found a significant difference. The mean ± SD percentage of Treg cells was 1.34 ± 0.74 pretransplant. This percentage increased significantly 3 months after MSCT (2.03 ± 0.80; n = 16) (P = 0.03) and continued to increase to 2.70 ± 1.70 at 6 months (P = 0.04; n = 5) (Figure 4A).

Recent studies have identified several cytokines related to Treg cell proliferation and differentiation. The most relevant are considered to be TGFβ and IL-10. We measured the changes in the concentrations of these 2 cytokines and found that the concentration of TGFβ increased significantly 3 months after MSCT (3.06 ± 1.60 ng/ml versus 1.96 ± 0.98 ng/ml at baseline; n = 13) (P = 0.003) and increased after 6 months (2.01 ± 1.59 ng/ml versus 0.92 ± 0.43 ng/ml; n = 3) (P = 0.07) (Figure 4B), while no significant changes were found in IL-10 concentrations (Figure 4C).
with the up-regulation of IFNγ at the same visit (38.38 ± 17.84 pg/ml versus 32.05 ± 19.10 pg/ml at baseline), although this difference was not statistically significant (P = 0.73).

Adverse events. Severe nausea occurred in 1 of the 16 patients during CYC infusion but disappeared immediately. No treatment-related mortality or other adverse events occurred during or after UC MSCT, and UC MSCT was well tolerated by all patients.

DISCUSSION

MSCs are multipotent, nonhematopoietic progenitor cells that are being explored as a promising new treatment for tissue regeneration. Although their immunomodulatory properties are not yet completely understood, their low immunogenic potential, together with their effects on immune responses, make them a promising therapeutic tool for severe refractory autoimmune diseases. MSCs have already been used in clinical trials as a treatment for acute graft-versus-host disease following allogeneic HSCT (25,26) and for autoimmune diseases such as systemic sclerosis (27) and type 1 diabetes mellitus (28).

In mice with lupus-like manifestations, heterogenic MSC, including BM and UC MSCs, is required for sustained improvement (refs. 22, 23, and Sun L, et al: unpublished observations). In 2007, we used UC MSCs to treat 2 severely affected patients with refractory SLE. Surprisingly, the disease activity was significantly improved by 3 months of followup, as manifested by amelioration of fatigue, proteinuria, ascites, and arthralgia in both patients, and resolution of seizures, nasal hemorrhage, and accelerated hypertension in 1 of them. Organ function improved notably, allowing tapering of prednisone and discontinuation of immunosuppressive drugs. After nearly 2 years of followup, the disease in these 2 patients was still in clinical remission, and the patients had not experienced any adverse events. We then began to enroll additional patients with treatment-refractory SLE in our protocol. In this larger-scale clinical trial, additional significant disease amelioration was noted, including decreases in 24-hour proteinuria and serum creatinine levels, along with increases in serum albumin, complement C3, and hemoglobin levels and platelet counts.

We used a preconditioning regimen of intravenous infusion of CYC (0.8–1.8 gm) in 11 patients to suppress overactive immunity. The disease in all of these patients was unresponsive to CYC or other immunosuppressants before MSCT. The dose of CYC used in our protocol was much lower than that used in autologous HSCT (5,29). There were no adverse events associated with the preconditioning regimen, and it was well tolerated. (Patient 2 had transient nausea and recovered rapidly.) No severe leukopenia or agranulocytosis was found. Five patients with severe cytopenia or myelo suppression did not receive the preconditioning regimen. We found no significant differences among patients who received the preconditioning regimen and those who did not receive the preconditioning regimen in terms of disease remission, suggesting that the treatment effect was attributable to MSC and not to CYC.

The improvement in disease activity and long-term normal levels of clinical markers and serologic features indicated that UC MSCT may suppress the underlying immune perturbations and fundamentally change the immune system. To further investigate the mechanism by which MSC might ameliorate the underlying pathogenesis of SLE, we assessed whether T cell abnormalities associated with lupus would normalize after transplantation.

Treg cells, a T cell subset differentiated from naive CD4+ T cells, have been studied in recent decades for their immunosuppressive activity in the control of immunity (30,31). Recent research has confirmed that the frequency and function of Treg cells are deficient in patients with active SLE (32) but could be restored by corticosteroid treatment inducing disease remission (33), suggesting a role for this T cell subset in the pathogenesis of human SLE. The transcription factor FoxP3 acts as a crucial molecule in Treg cell development and function and is widely used as a nuclear marker for Treg cells (34). In this study, we observed that UC MSCT could markedly up-regulate the percentage of CD4+FoxP3+ T cells in peripheral blood mononuclear cells 3 months after transplantation, and the percentage continued to increase after 6 months. In addition, the restoration of Treg cells was associated with a concomitant increase in TGFβ and, to a lesser degree, an increase in IL-10, 2 cytokines that play important roles in Treg cell activation and function (35,36). Augmentation of the Treg cell pathway may be one of the mechanisms underlying the therapeutic effect of UC MSCs.

IFNγ and IL-4 are 2 typical cytokines representing Th1 and Th2 subsets. Schwarting et al (37) found that IFNγ protected against advanced SLE progression. On the other hand, IL-4 levels were found to be elevated in the peripheral blood of patients with active SLE (38). Such results suggest that the balance of Th1 and Th2 in SLE is shifted toward to Th2. In the present study, UC
MSCs significantly depressed the serum concentration of IL-4 3 months after transplantation, which may have inhibited humoral immunity in the SLE patients and indirectly suppressed the secretion of autoantibodies, especially anti-dsDNA antibody (which is most closely correlated with clinical outcome in mice and humans with SLE [39]). In addition, it has been shown that MSCs exert a suppressive effect on B cell activation and terminal differentiation to plasma cells (40); therefore, suppression of humoral immunity may be one mechanism involved in the treatment effect of UC MSCT.

However, for 2 patients with nephritis in the present study (patients 6 and 12), renal function improved significantly, while there was little change in serum anti-dsDNA antibody level at the 3-month visit, suggesting that in addition to the inhibition of the secretion of autoantibodies by activated plasma cells, immunosuppressive properties possessed by UC MSCs are also involved in preventing recurrence. Serum C3 levels were normal even 12 months after MSCT in patients 1 and 2, in parallel with systemic remission, suggesting a long-term effect of UC MSCs on these patients.

In conclusion, we provide direct evidence that UC MSCT exerts a profound therapeutic effect in patients with severe and refractory SLE. At least 3 months of clinical and serologic improvement were achieved in all patients, and in 2 patients, this was achieved without any immunosuppressive drugs. This is the first study to demonstrate that UC MSCT is safe and effective, at least in the short term, in treating patients with severe SLE. Further clinical trials with more patients, longer periods of followup, and comparisons with standard treatment will be needed to determine the efficacy and safety of this novel approach to the treatment of lupus.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

Study conception and design. Sun, Dandan Wang, Liang, Zhang, Feng, Hong Wang, Hua, Liu, Ye, Hu, Xu, Zeng, Hou, Gilkeson, Silver, Lu, Shi.

Acquisition of data. Sun, Dandan Wang, Liang, Zhang, Feng, Hong Wang, Hua, Liu, Ye, Hu, Xu, Zeng, Hou, Gilkeson, Silver, Lu, Shi.

Analysis and interpretation of data. Sun, Xu, Zeng, Hou, Gilkeson, Silver, Lu, Shi.

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