


Safety, immunological effects and clinical response in a phase I trial of umbilical cord mesenchymal stromal cells in patients with treatment refractory SLE

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To cite: Kamen DL, Wallace C, Li Z, *et al.* Safety, immunological effects and clinical response in a phase I trial of umbilical cord mesenchymal stromal cells in patients with treatment refractory SLE. *Lupus Science & Medicine* 2022;**9**:e000704. doi:10.1136/lupus-2022-000704

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/lupus-2022-000704>).

Received 4 April 2022
Accepted 23 June 2022



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ABSTRACT

Background Reports of clinical improvement following mesenchymal stromal cell (MSC) infusions in refractory lupus patients at a single centre in China led us to perform an explorative phase I trial of umbilical cord derived MSCs in patients refractory to 6 months of immunosuppressive therapy.

Methods Six women with a SLEDAI >6, having failed standard of care therapy, received one intravenous infusion of 1×10^6 MSCs/kg of body weight. They maintained their current immunosuppressives, but their physician was allowed to adjust corticosteroids initially for symptom management. The clinical endpoint was an SRI of 4 with no new British Isles Lupus Activity Guide (BILAG) As and no increase in Physician Global Assessment score of >0.3 with tapering of prednisone to 10 mg or less by 20 weeks.

Results Of six patients, five (83.3%; 95% CI 35.9% to 99.6%) achieved the clinical endpoint of an SRI of 4. Adverse events were minimal. Mechanistic studies revealed significant reductions in CD27IgD double negative B cells, switched memory B cells and activated naïve B cells, with increased transitional B cells in the five patients who met the endpoint. There was a trend towards decreased autoantibody levels in specific patients. Two patients had increases in their Helios+Treg cells, but no other significant T cell changes were noted. GARP-TGF β complexes were significantly increased following the MSC infusions. The B cell changes and the GARP-TGF β increases significantly correlated with changes in SLEDAI scores.

Conclusion This phase 1 trial suggests that umbilical cord (UC) MSC infusions are very safe and may have efficacy in lupus. The B cell and GARP-TGF β changes provide novel insight into mechanisms by which MSCs may impact disease.

Trial registration number NCT03171194.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Mesenchymal stromal cells (MSCs) have significant immune modulatory effects. There are to this point primarily small case series and uncontrolled trials of MSCs in lupus, almost all are from China. Thus, the efficacy of MSCs in lupus is unknown overall and safety unproven in non-Asian patients.

WHAT THIS STUDY ADDS

⇒ This study adds assessment of MSC safety and immunological activities in a mixed ethnic cohort. The B cell and GARP changes following MSC infusion are novel and previously unreported. Although no efficacy assessments can be made from this phase I trial, the lack of attributable adverse events and the achievement of an SRI4 in 5/6 patients supports the need for further assessments of efficacy and toxicity in a larger controlled trial.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ If further controlled trials show efficacy, given their safety profile, MSCs would be a new therapeutic approach to treating lupus with a better safety profile compared with current therapies. The extensive immunological studies also provide important insight into lupus pathogenesis and mechanisms of action of MSCs on the immune system.

INTRODUCTION

SLE is a heterogeneous disease affecting young women in their childbearing years.¹ The hallmark of disease is production of autoantibodies with immune complex deposition in target organs. Despite research progress and recent clinical trials' success, there is still a need for more effective safe treatments.^{2 3} Current immunosuppressive and biological therapies have therapeutic effects, yet a significant number of lupus

patients remain inadequately responsive to current therapies. An additional issue with current therapies is the side effect profile especially for women of childbearing potential.⁴ Cellular therapies, such as mesenchymal stromal cells (MSCs) are an emerging area of interest as to their therapeutic efficacy in immune diseases including lupus.

MSCs are derived from bone marrow, adipose tissue and umbilical cords/placentas.^{5–7} Their local autologous use in plastic and orthopaedic surgery is proven beneficial.^{8,9} There is growing literature on the immune properties of MSCs and their use in immune-mediated diseases.^{10,11} Trials of MSCs in refractory graft versus host disease (GvHD), rheumatoid arthritis, inflammatory bowel disease and lupus have had variable results.^{12–17} Most were uncontrolled trials with small numbers of participants. There is a benefit of MSCs for steroid refractory paediatric GvHD¹⁸ and local use in healing fistulas in Crohn's disease.¹⁹ The efficacy in other diseases remains unproven due to a lack of placebo-controlled trials.

There are a number of publications regarding use of MSCs in refractory lupus from a single centre in Nanjing, China.^{15, 20, 21} The reports provide both short-term and long-term follow-up of dozens of patients treated with MSCs. There was an overall response rate of 60%–65% at 6 months following a single MSC infusion of 1 million cells per kilogram. Long-term beneficial effects on disease activity were reported.^{15, 21, 22} The patients primarily had lupus nephritis, but other manifestations of lupus were also improved. The length of response varied from 6 months to 5 years.²³ None were placebo controlled. The one controlled trial of MSCs in lupus nephritis, enrolling treatment naïve patients, was small (18 patients) and based on a high response rate to cyclophosphamide alone versus cyclophosphamide plus MSCs did not detect an added benefit of MSCs.²⁴

MSCs advantages are they are easily obtained, have a low side effect profile and can be given without histocompatibility matching or preconditioning.²⁵ The reported 'immune privilege' of MSCs is based on their not expressing MHC Class II or immune cofactors, rendering them initially hidden from the host immune system.²⁶ There are literally dozens of proposed mechanisms for the immune effects of MSCs, though none are proven in humans.^{27, 28}

Due to the promising results out of China, we initiated studies of MSCs in lupus, starting with murine models, that demonstrated efficacy of MSCs from human controls in reducing renal disease.²⁹ Allogeneic MSCs were used in this phase I trial as autologous MSCs from lupus patients are not as immune active as allogeneic MSCs.³⁰ In a limited study of autologous bone marrow MSCs, two lupus patients did not have a beneficial effect on their disease.³¹ Based on the lack of definitive evidence of MSC efficacy and lack of safety data in non-Asian patients, we performed a phase I safety trial in treatment refractory lupus in a multiethnic cohort as a stepping stone to a larger phase II efficacy trial. Assessing safety and potential

mechanistic effects are an important first step in development of a new therapy.

METHODS

Preparation of UC MSCs

The MSCs were derived from UCs of two healthy donors under FDA IND 16377. The donors were mothers in the OB/GYN clinic undergoing elective C-sections. After informed consent, the mother's blood was tested using the infectious testing battery required for allogeneic bone marrow donors within 1 week of delivery. ANA screening was done. Potential donors were excluded if they had personal or family history of autoimmune disease or any positives on infectious and autoimmune testing of their blood. The cords came from one male and one female infant. The UCs were obtained using sterile technique and transported to the MUSC Center for Cellular Therapy. The derived cord cells were plated and incubated in a minimal essential medium (MEM, GIBCO) with glutamine and 10% sterile pooled human platelet lysate (Cook Regenec Inc). Aliquots were tested for bacterial, fungal, endotoxin and mycoplasma. MSC immune potency was measured by T cell proliferation and interferon-gamma induced IDO expression. Further details are provided in the online supplemental methods.

Patients

Patients had a historical presence of at least 4 of 11 of the ACR Lupus Classification Criteria.³² Further inclusion criteria included: age between 18 and 65 years old, either sex, any race, evidence of a positive ANA ($\geq 1:80$ titre) or positive dsDNA antibody test within 6 months of screening, clinically active SLE determined by SLEDAI score ≥ 6 and ≤ 12 and the presence of one BILAG A or one BILAG B at screening, despite standard of care (SOC) therapy. If the BILAG A or the BILAG B was in the renal organ system, the patient must have completed 6 months with either mycophenolate mofetil or cyclophosphamide. Non-nephritis patients had active disease despite 3 months of SOC therapy. Patients were able and willing to give written consent. Details regarding patient selection are in the online supplemental methods and in [table 1](#). A sample size of 6 was selected in an attempt to balance the need to investigate the safety of this therapy with the need to limit any negative consequences should they occur.

Clinical endpoints

Clinical response

The SLE Responder Index (SRI) 4 was used as the assessment tool for clinical activity. A decrease in the SLEDAI of at least 4, no new BILAG As or two BILAG Bs and no increase in the Physicians Global Assessment >0.3 were required to be considered responsive. This assessment was made at weeks 0, 4, 8 and 24 after the MSC infusion. The week 24 assessment was the primary endpoint. Inability to taper prednisone to 10 mg or less by 20 weeks was considered a treatment failure. Dose increases or new

Table 1 Safety reports during the trial including AEs and the one SAEs

Subject	Non-serious AEs (#NCI-CTCAE Grade)	SAE (#)	AEs related to MSCs	Early withdrawal
1	3 grade 1 5 grade 2 0 grade ≥3	0	Grade 2 nausea ('possible')	
2	0 grade 1 2 grade 2 0 grade ≥3	0		
3	2 grade 1 2 grade 2 0 grade ≥3	1	Grade 1 paraesthesias ('possible') Grade 1 flushing ('possible')	Dropped out after week 8 visit
4	0 grade 1 3 grade 2 0 grade ≥3	0	Grade 2 tachycardia ('possible')	
5	1 grade 1 1 grade 2 0 grade ≥3	0		
6	1 grade 1 1 grade 2 0 grade ≥3	0		
Total	21 AEs	1 SAE	4 AEs possibly treatment related; all resolved without sequela	1 early withdrawal to pursue other SLE treatments at week 8

There were no grade 3 or higher AEs. AEs deemed definitely not related to the investigational product are totalled numerically but not detailed in the table. Attribution of the AEs and SAE is presented in column 3.

AEs, adverse events; MSCs, mesenchymal stromal cells; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; SAE, serious adverse event.

additions to SOC immunosuppressant therapy for SLE activity prior to week 24 were considered a treatment failure. Secondary outcomes included the SF-36 (short form 36) quality of life instrument and the Lupus Impact Tracker.³³

Safety

Study participants reported adverse events (AEs) throughout the trial, regardless of attribution. Lowering of SOC immunosuppressant therapy due to toxicity was allowed. Further safety methodology is in the online supplemental methods.

Treatment protocol

All patients received UC-derived MSCs suspended at a concentration of 2×10^6 cells/mL in Plasma-Lyte A (Baxter) suspension media. The patients and the treatment team were aware they were all receiving MSCs. The patients received 1×10^6 cells/kg body weight. The infusion rate was 100×10^6 over 10 min. Patients received premedication of Benadryl 25 mg and 650 mg of Tylenol orally. There was no preconditioning or Human Leukocyte Antigens (HLA) matching. If the patient was cytomegalovirus (CMV) antibody negative, they received cells from the donor that was CMV negative. If the patient was CMV antibody positive, but not having an acute infection, they received cells from the CMV positive donor. Further description of the treatment protocol is in the online supplemental methods.

Statistical analysis

The primary endpoint consisted of the proportion of participants who exhibited a clinical response at week 24 by SRI 4. This proportion was reported along with an exact 95% CI. All secondary analyses were conducted in an exploratory fashion with p values and CIs presented without adjustments for multiple comparisons. Interval estimates were generated at the 95% confidence level.

Since many of the secondary endpoints were collected at multiple time points, statistical models appropriate for longitudinal data analyses were used.³⁴ General linear mixed models (GLMMs), including appropriate covariance structures to account for within-subject clustering, were constructed for the different outcomes to determine whether there were significant changes over time (ie, for the SLEDAI, SF-36 and Lupus Impact Tracker (LIT)) and whether certain outcomes were correlated with others (ie, B/T cell subtype distributions and autoantibody levels). Sensitivity analyses were conducted by adopting a last observation carried forward (LOCF) approach within the GLMM models, given that one subject (#3) did not contribute data after week 8.

Mechanistic studies

Protocols for handling of specimens, B cell and T cell characterisations, ELISA assays and glycoprotein A repetition predominant (GARP) assays are in the online supplemental methods section.

Table 2 Baseline demographics and disease characteristics in the six participants

Subject	Age, race/ethnicity sex	Baseline SLE manifestations	SLE duration at baseline	Baseline SLE medications	Baseline SLEDAI score	Week 24 SLEDAI score
1	30–35 year old female	Transverse myelitis, alopecia, oral ulcers, pleuritis	4.8 years	HCQ, MMF, prednisone	6	0
2	35–40 year old female	Arthritis, alopecia, oral ulcers	9.9 years	HCQ	8	2
3	30–35 year old female	Arthritis, alopecia, peritonitis, angioedema	10.5 years	Cyclosporine, prednisone	6	NA
4	25–30 year old female	Rash, oral ulcers, alopecia, low complement, +dsDNA abs, nephritis	11.7 years	HCQ, MMF, prednisone	10	6
5	45–50 year old female	Rash, arthritis, oral ulcer	8.7 years	HCQ, prednisone	8	0
6	35–40 year old female	Arthritis, low complement, +dsDNA abs, leucopenia	3.9 years	HCQ, MMF, AZA, prednisone	11	5

Demographics of the participants is presented in column 2 with baseline lupus manifestations and disease duration are shown in columns 2 and 3. There was a wide range of disease manifestations and disease duration. Baseline medications, SLEDAI at baseline and SLEDAI at week 24 are presented in columns 5, 6 and 7.

AZA, azathioprine; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil.

RESULTS

Safety

As shown in [table 1](#), there were a total of 21 AEs over the 52 weeks of the trial. None were grade 3 or higher, and only four were felt possibly related to the MSC infusions and all resolved quickly. These included mild nausea, paraesthesias and flushing. There were no lab-related AEs in the six participants. One patient dropped out (patient 3) and was treated with rituximab by her primary rheumatologist 4 months post-MSC infusion for refractory symptoms. Her SAE was an anaphylactic reaction to the rituximab. She lived in California and did not want to make the cross-country trips post-week 8. This was the only SAE in the trial and was judged not due to MSC treatment given a prior history of multiple anaphylactic reactions to intravenous medications. There were no other common AEs within the group.

Clinical response

The six patients enrolled were female with an average age of 38 years (range of 26–48 years). Two participants were African-American, one was Hispanic and three were Caucasian. Average disease duration was 8.2 years (range of 3.9–11.7 years). One patient had onset of disease as a child. Baseline disease features are in [table 2](#). One patient had refractory episodes of transverse myelitis despite immunosuppression and biological therapy. One patient had renal disease with ongoing proteinuria post-therapy with mycophenylate (MMF). All but one patient were on hydroxychloroquine and prednisone. Two were on MMF, one on azathioprine and MMF and one on cyclosporine. Two were not on an immunosuppressant having failed multiple immunosuppressive regimens. All patients continued their baseline medications throughout the trial.

Overall, 5 (83.3%; 95% CI 35.9% to 99.6%) of the six participants receiving UC MSCs reached the primary response criteria of an SRI of 4 by 24 weeks and a decrease in prednisone to 10 mg a day or less by 20 weeks ([figure 1A](#), [table 3](#) and online supplemental table 1). By

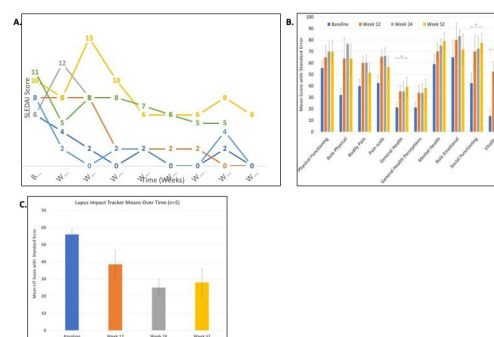


Figure 1 Clinical and patient-reported outcomes. (A) Line plot of the change in SELENA-SLEDAI scores over the course of the trial. This is the overall score combining lab and clinical criteria. The primary endpoint was the SLE Responder Index (SRI) 4 at 24 weeks. There was overall a significant decline in SELENA-SLEDAI scores of 5/6 patients meeting the SRI-4 endpoint ($p < 0.006$). (B) Lupus Impact Tracker (LIT) means over the time of the study. There was a significant decrease in the LIT beginning at week 12 and continuing through week 52 ($p = 0.007$). Error bars indicate SD. Represents the scores of the five patients completing the trial to 52 weeks. (C) SF-36 subscale scores at the given timepoints for the five patients who completed the study. Significant increases in general health ($p = 0.02$), social functioning ($p = 0.02$) and vitality ($p = 0.004$) were present beginning at week 12 and continuing through week 52. Data represents the scores of the five patients completing the trial to 52 weeks. SELENA SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment Systemic Lupus Erythematosus Activity Index.

Table 3 Change in the physician global assessment and prednisone dosing

Subject	Physician's Global Assessment (PGA) change over 24 weeks (scale 0–3)	Baseline prednisone dose (mg/day)	Week 24 prednisone dose (mg/day)	Week 52 prednisone dose (mg/day)
1	–1.94	10	7.5	5
2	–0.9	0	0	0
3	n/a	20	–	–
4	–1.23	20	10	2.5
5	–1.05	10	10	10
6	–1.8	10	10	10

The change in the PGA from baseline to week 24 is presented in column 2, while the change in prednisone dosing from baseline to week 24 and to week 52 is presented in columns 3 and 4. Three of the five responders were able to taper prednisone to 5 mg or less, while two maintained their prednisone dose at 10 mg/day.

week 24, results from the GLMMs showed that there was a significant ($p < 0.001$) decline from baseline in the SLEDAI scores, decreasing from a baseline average of 8.2 (range 6–11) to 2.8 (range 0–6) at week 24, for a mean decline of 5.3 units (95% CI 2.7 to 8.0). Significant ($p < 0.05$) and sustained responses in the SF-36 scores and the LIT over time were observed (figure 1B and C). Sensitivity analyses using the LOCF approach for missing data yielded results that were similar to the primary analyses; significance ($p < 0.05$) was preserved for the time changes noted in the SLEDAI; the SF-36 general health, social functioning and vitality domains; and the LIT.

Standard laboratory measures were assessed. Participant 3's proteinuria improved from baseline (1015 mg/g/day) to week 24 (192 mg/g/day) with an increase in her lymphocyte count from 290 to 1000 and her C4 from 9.5 mg/dL to 13.1 mg/dL. Participant 6 had a decrease in her anti-dsDNA level from a baseline of >300 IU/mL to 132 IU/mL at week 24. None of the other patients had low complement levels or high anti-dsDNA levels.

As shown in online supplemental figure 1A, titres for anti-Ro52, anti-Ro60, anti-Sm and anti-RNP were assessed at each of the 0-week, 4-week, 8-week and 24-week time-points. All the participants had increased titres of anti-Ro52, anti-Ro60 and anti-RNP greater than control. Participants 5 and 6 had titres of anti-Sm elevated above control. In participants 2 and 6, there was a log-fold decrease in anti-Ro60 antibodies between week 0 and week 4 that remained through week 24. Other titres remained stable over the time of the study. The assay for these measures is the LIP assay, which has a broader dynamic range than standard ELISA measures perhaps explaining the changes seen in these assays over time.

There was stability or further improvement in the clinical response from 24 to 52 weeks in the five responders (figure 1A and online supplemental table 1). Physician global and patient global assessments were significantly improved in the 5/6 responders (table 3 and data not shown). Prednisone was able to be tapered or maintained at 10 mg or less per day (table 3). There was a sustained response in the LIT (figure 1C). Subsequently, from 18

to 48 months after completion of the study, four of the patients flared. Patient 1 had a recurrence of her thoracic cord transverse myelitis at 20 months postinfusion. She was treated with Cytoxan and pheresis and retreated with MSCs. She has had no flares now 34 months later. The other three participants had less severe flares with arthritis and skin disease and were not retreated with MSCs.

Mechanistic studies

B cell responses

Flow cytometry was performed on patient samples at weeks 0, 4, 8 and 24. Week 8 data are not included due to weather induced loss of 3 week 8 samples in transit. The gating scheme was previously published with the identification of nine B cell subsets³⁵: (1) plasmablasts, (2) double negative 3+4, (3) double negative 2 (DN2), (4) double negative 1, (5) switched memory (SM), (6) unswitched memory, (7) activated naïve (aN), (8) resting naïve+transitional 3 and (9) transitional 1+2 (T1+T2).

As shown in figure 2, there was variation in percentage of B cell subsets at baseline. A significant change from week 0 to 24 was a marked decrease in the percentage of total DN B cells in participants 1, 2, 5 and 6 (figure 2 and online supplemental table 2). DN2 B cells are expanded in African-American women with active lupus.³⁵ Of interest, the two patients with the highest numbers of DN2 cells at baseline were the two African-American participants (1 and 6). There was a significant reduction in DN2 B cells following MSC infusion. aN B cells are also increased in African-American females with lupus.³³ Three of the participants (1, 5 and 6) had detectable numbers of aN B cells. Participants 1 and 6 had expanded DN2 B cells and aN B cells in parallel through the study. Patients 2 and 5 were not on immunosuppressants at the time of study entry, yet still had significant changes in their B cell profiles similar to the patients on immunosuppressants.

Concomitant with the decrease in DN and aN B cells, there was an increase in resting naïve and T1+T2 B cells. There was a significant change in the SM B cells, decreasing in all five of the patients that were responders. Online supplemental table 1 presents the B cell data in a

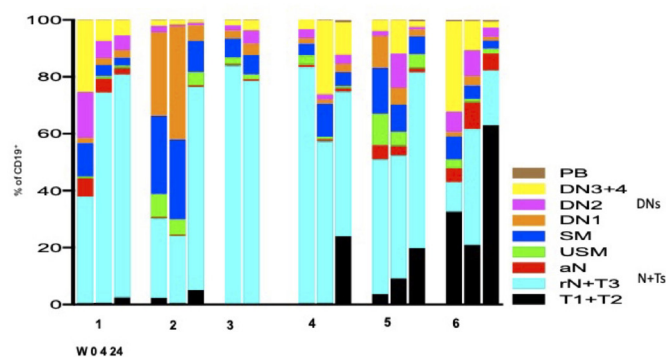


Figure 2 B cell subset changes over time by flow cytometry. (B) Cell subset changes over time as determined by flow cytometry. Significant differences in B cell subsets occurred over the trial period as also noted in online supplemental table 2. The cell markers used are listed in the methods and are previously published.³³ Most significant changes were in decreased double negative B cells including DN2, increased transitional B cells and decreased activated naive B cells. IgD⁻CD27⁺=SM+PB, IgD⁻CD27⁻=DN1+DN2+(DN3 +4), IgD⁺CD27=(T1 +T2)+(rN +T3)+aN. aN, activated naive; DN, double negative; rN, resting naive; SM, switched memory; T, transitional; PB, plasmablasts; USM, unswitched memory.

numerical format including the SLEDAI score calculated at the week 0-week, 4-week and 24-week visits. There were significant associations between subjects' SLEDAI scores and their percentages of N+T ($p=0.042$), SM ($p=0.007$) and DN ($p=0.041$) B cells over time. There was a negative correlation for N+T with the SLEDAI and positive correlation of the SM and DN with the SLEDAI.

T cell responses

Prior studies of MSC infusions in lupus-prone mice, and in more limited studies in human lupus, reported an increase in Treg cells with a decrease in Th17 and T follicular helper (Tfh) cells following MSC infusions.³⁶

As shown in figure 3A, we assessed the fold change of Treg cells in the peripheral blood of the participants. In patient 1, there was a significant increase in percentage/fold change in Treg cells. This change was present in both Helios⁻ and Helios⁺ Treg cells. Participant 6 also had an increase in Treg cells present only at week 24 and primarily in Helios⁻ Treg cells. Participant 2 had a consistent increase in her Tregs as measured by fold change compared with baseline though percentage change was small.

Online supplemental figure 1B demonstrates there were fluctuations in Th1, Th2 and Th17 cells but no clear trends during the study. In online supplemental figure 1B, we also present measures of Tfh and T peripheral helper (Tph) cells. As expected, there were very few Tfh cells detected, although participant 1 had a sustained decrease following MSC infusion. A limited number of Tph cells were detected, and there was no significant change during the study. There were no associations between SLEDAI score and changes in T cell subsets other than in Treg levels in patients 1 and 2. There were no detectable

changes in CD8⁺ T cells or their subsets, nor NKT cells (data not shown).

GARP is a cell surface protein that is a repository for latent TGF β and plays a key regulatory/tolerance role in immunity via modulating TGF β activation.³⁷ It is primarily expressed on platelets, Tregs, activated B cells and MSCs.^{38,39} Lack of GARP expression on murine B cells or Treg cells resulted in lupus-like autoimmunity.³⁹ We postulated that GARP was involved in the impact of MSCs on the immune response. We assessed the presence of soluble GARP-TGF β complexes in the serum of lupus patients from our biorepository that were not in the trial. There was a significant ($p=0.023$) decrease in serum levels of circulating GARP-TGF β complexes in lupus patients versus controls (figure 3D). We then assessed if there was a correlation between circulating GARP-TGF β levels and disease activity in these biobank patients. As shown in figure 3E, there was a significant inverse correlation ($p=0.034$) between serum levels of soluble GARP-latency-associated peptide (LAP) and SLEDAI scores in patients with active disease (SLEDAIs >10). In figure 3E, prior to infusion, serum GARP levels were undetectable in the six participants. At week 4, GARP-TGF β serum levels were significantly increased from baseline in all patients. At week 8, levels fell in all the patients but remained above baseline. At week 24, there was an upward rebound or stability of GARP levels in four of the five patients that completed the study (1, 2, 3 and 5).

DISCUSSION

This phase I trial is the first of allogeneic MSCs performed in multiethnic lupus patients. The results indicate that infusion of allogeneic UC MSCs appears safe short term, as we had no serious adverse events that were attributed to the UC MSC infusions, and all the AEs were grade 2 or less. We were encouraged that five of the six patients treated met the primary endpoint of an SRI 4, justifying performing the phase II double-blind multicentre efficacy study currently in progress. We were also encouraged by the marked B cell changes and increased serum GARP-TGF β measures noted indicating the MSCs had a systemic immune effect paralleling the clinical effects.

The patients in this trial were of mixed ethnicity with a range of ages. The patients had variable lupus manifestations. The prior studies in China by Dr Sun included patients with primarily refractory lupus nephritis, but also included patients with significant hematological involvement and pulmonary haemorrhage.^{15,21,22} In a recent review of his cohort, Dr Sun reported that younger male patients and those with musculoskeletal symptoms were not as responsive to UC MSC infusion as other lupus manifestations.²⁰

The duration of response is variable in the reports from Dr Sun's group.^{15,21,22} He reported a 65%–70% early 'response' rate with a long-term response rate of 4–5 years in the 25% range. A limited number of patients in his cohort are beyond 5 years with minimal disease activity.

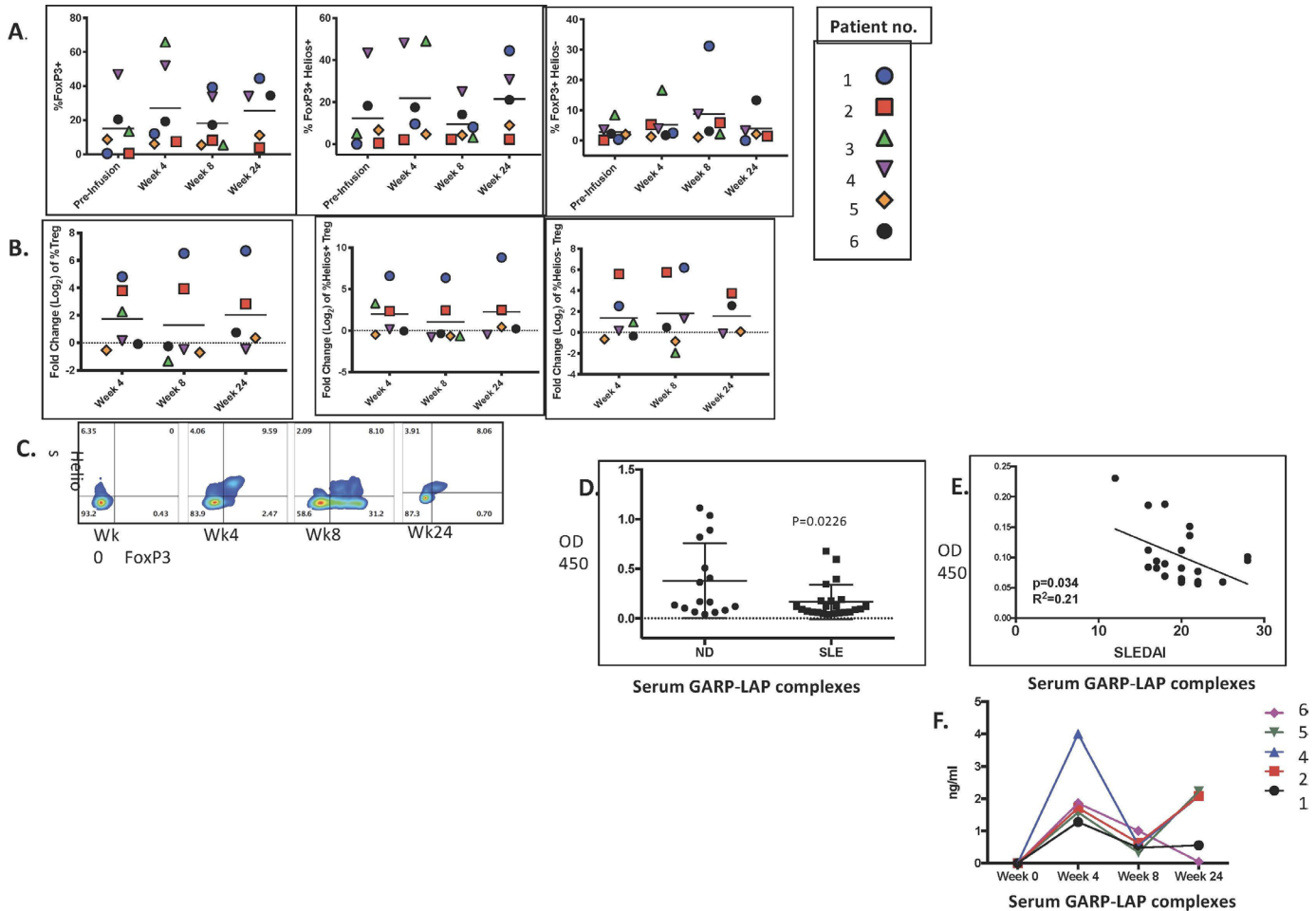


Figure 3 Cell changes over time. (A) Treg changes over time are presented. Participant 1 had a significant increase over time in Tregs both Helios+ and Helios- looking at percentage change and fold change over baseline. Participant 2 had a significant increase with time compared with baseline in both Treg subsets, though the percentage change was small given the low percentage of Tregs at baseline (B). A representative flow block is shown in figure part C demonstrating the increase in Tregs over time from 0% to 8% in participant 1. (D–F) GARP serum measures. Figure part D shows measures of circulating serum GARP-LAP (latency-associated peptide) complexes as measured by a sandwich ELISA assay as previously described.³⁷ Random SLE patients in the MUSC cohort (n=30) had significantly less ($p=0.0226$) circulating GARP-LAP complex than age/sex/race matched controls (n=16) y-axis is OD 450. (E) Plots GARP-LAP complexes via ELISA versus patient SLEDAI score at the time of blood draw (n=21) for the MUSC lupus cohort. The y-axis is OD 450 reading via ELISA. There is a significant correlation between SLEDAI score and GARP-LAP complexes in patients with SLEDAI scores >10. Figure part F is a sandwich ELISA measure of soluble serum GARP expressed as ng/mL of serum in the MSC treated patients over time demonstrating near 0 levels of serum GARP at baseline with significant increases at week 4, decreasing at week 8 prior to rebounding at week 24 in 3/5 patients. Mann-Whitney U test was used to determine significance (week 0–week 4– $p=0.003$). GARP, glycoprotein A repetition predominant; LAP, latency-associated peptide; MUSC, Medical University of South Carolina; MSC, mesenchymal stromal cell; OD, optical density.

Of the five ‘responsive’ patients in our trial, one remains with minimal disease activity out 3–4 years from their one-time infusion (participant 6). The other four patients had a full or partial flare of their disease from 18 months to 30 months post-single infusion. The response of participant 1 to retreatment was consistent with prior data from Dr Sun’s group reflecting retreatment is often successful.

There are prior reported trials of MSCs in human lupus; seven used allogeneic derived cells and one used autologous bone marrow-derived cells.^{31 40–42} All but two trials were done in China. There is only one ‘placebo-controlled trial’ of MSCs in lupus nephritis patients that were new onset and untreated comparing MSCs plus

cyclophosphamide to cyclophosphamide alone.^{24 43} The other two reports of MSCs in lupus were case reports from Europe, one using autologous cells that showed no improvement.⁴⁰ A more recent paper described compassionate use of UC MSCs in three women with class IV lupus nephritis.⁴³ They reported a complete remission in two patients and a partial remission in a third.

The only large placebo-controlled trials reported to date of MSCs in allo and autoimmune diseases used MSCs for treating Crohn’s disease and GvHD. The method of derivation and validation of the cells were not described. The MSCs however were late passages and were infused post-thawing, both of which are known to impact MSC

functionality. These trials showed trends towards efficacy but did not meet their target endpoint. These failures are likely due to the quality of the cells but led some to postulate MSCs are not effective in immune-mediated diseases.⁴⁴ There is demonstrated efficacy of MSCs in treating steroid refractory GvHD in paediatric patients receiving allogeneic bone marrow. MSCs are approved to treat GvHD in paediatrics in Canada, Japan and New Zealand. MSCs given by direct injection into the local area is approved to treat refractory fistulas in Crohn's patients in the European Union.

Patients in our trial were on hydroxychloroquine, prednisone and different immunosuppressants. Due to lack of response, two patients had their immunosuppressants discontinued prior to entry into the study. In this limited series, nor in the Sun trials, was there any indication of effects of concomitant medications on responses to MSCs. The impact of concomitant medications on response to MSC therapy is unresolved though data to date suggest a minor if any effect.

There are a number of unanswered questions regarding UC MSCs in lupus. The first is how variable are UC MSCs between donors in their efficacy. In this series, we did not see a differential response between the recipients of the two different cord cell lots. In our preclinical studies in lupus-prone mice, we used four different bone marrow donors from controls and three from lupus patients.²⁹ The MSCs from controls were more effective in preventing disease progression in the mice than were lupus-derived MSCs. The lupus-derived MSCs had induced indoleamine 2,3-dioxygenase (IDO) expression by gamma interferon and suppressed stimulated T cell proliferation similar to cells from controls. They were, however, not as effective in preventing B cell proliferation.²⁹ Given the prevalence difference in men versus women, one could speculate MSCs derived from males would be more effective than females. We have insufficient numbers to suggest there are differences in MSCs depending on sex of the donor. Defects in lupus MSCs are increasingly reported in vitro^{45–47}; definitive studies of differences in in vivo efficacy of different MSC preparations are lacking. Studies comparing bone marrow-derived versus adipocyte-derived versus UC-derived suggest subtle differences in function, but no definitive data that one source is superior in in vivo trials in humans. We used UC MSCs due to their ready availability, rapid growth characteristics and the ability to treat multiple patients with one cord (>90).

It is clear in humans that following intravenous infusion the majority of the cells are trapped in the lung, but how long they remain viable is unknown.⁴⁸ It is not known whether the MSCs have to migrate to the affected organ for cell-to-cell interactions for effect or if MSC derived endosomes/cytokines are sufficient.^{49 50} In studies in mice infused with human cells, there are reports of a short half-life for the MSCs, while others, including our group, found evidence of MSC survival in target organs for weeks postinfusion.²⁹ The only human study of MSC survival looked for HLA mismatched MSCs at autopsy of

patients having undergone MSC infusion for GvHD.⁵¹ MSCs could be detected in different organs weeks after the MSC infusion. Whether cells that are MHC matched or closely matched are preferable to mismatched cells is also unclear, though the 'immune privilege' reported for MSCs is time limited.⁵² Alloreactivity postinfusion is variable and may or may not enhance MSC effects.

As controversial as is the efficacy of MSCs, the mechanisms by which they impact disease is also unclear. In vitro data indicate that MSCs can suppress the activity of almost every immune cell, while enhancing regulatory B and T cells. A host of mediators are secreted by MSCs including IDO, NO, PGE2, TGF β , IL10, Factor H and hepatic growth factor.^{53–55} MSC cell surface molecules such as GARP and FLT3L are postulated to interact with host immune cells impacting proliferation, differentiation and activity.^{39 56} Others showed in mice that MSCs are engulfed by resident macrophages inducing a tolerogenic anti-inflammatory phenotype that prolongs the efficacy of MSCs. MSCs are ineffective in mice lacking macrophages.^{56 57} At the cellular level, as noted previously, MSCs are reported to increase Tregs and Bregs while decreasing Th17 cells, Tfh cells and inducing a Th1 to Th2 shift possibly via TGF β effects.⁵⁸ Enhancing development of CD1c+ tolerogenic dendritic cells (DCs) via expression of FLT3L by MSCs was reported, enhancing IFN γ production by CD8+ T cells.⁵⁶ Although all of the previously mentioned may contribute, the actual defining mechanisms remain unknown.

Although we did not note any significant changes in the T cell compartment other than in two patients, we did find marked changes in the B cell compartment. The significant effect on DN B cells, aN B cells and switched (SW) B cells was not reported in published MSC studies. The importance of these findings is supported by the correlation of SLEDAI scores and changes in B cell subsets. The DN B cells and activated naïve cells are increased in active lupus and are believed to be precursors of autoantibody producing cells in lupus.⁵⁹ Epigenetic analysis of these DN B cells in lupus patients revealed they are primed to respond to TLR ligands, especially TLR7.³⁵ The changes in ENA autoantibodies is of note, given the reported stability of these antibodies despite therapy. The technique used in this study was the LIP assay which has a broader detection range than standard clinical assays allowing better detection of changes in antibody levels.

It is unclear if the effect of MSCs on B cells is a direct effect or an indirect effect. The impact on B cells appears less likely to be due to T cell effects since we saw evidence of a T cell change in only two patients, while the B cell effect was present in all five responders. Based on the known expression of GARP on MSCs and a prominent role for GARP in tolerance and autoimmunity in mice,³⁹ we assessed GARP-TGF β levels in participants in this trial. When a GARP bearing cell interacts with another cell that expresses GARP, expression of GARP is increased on both cells.³² This affect may explain the rebound of GARP-TGF β levels at 24 weeks or may reflect the improvement

in disease activity in patients with resultant increased GARP-TGF β expression.

If future larger trials prove significant efficacy of MSCs in treating lupus, where would such treatment fit into the treatment algorithm of lupus. Producing the cells can be done given 90–100 patients can be treated with a single cord, and a number of companies and institutions are developing the ability to produce MSCs for human use. Given the safety, the ease of infusion and the length of response, if MSCs are shown to have equal or superior efficacy to current treatments, acceptance and use of MSCs in lupus will likely be broad.

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Acknowledgements We would like to acknowledge the patients that participated in the study, the MUSC Center for Cellular Therapy and the nurses in the MUSC Nexus Clinical Research Center.

Collaborators NA.

Contributors DLK was the clinical leader and designer for the trial and performed many of the study visits; CW was involved in mechanistic study design and performing the lycoprotein A repetition predominant (GARP) assays. ZL was involved in the design of the mechanistic studies including the GARP assays. MW was involved in designing the T cell studies and running the assays. CP designed the T cell studies and oversaw their completion. CW designed and oversaw the B cell mechanistic studies; HW was involved in study design and oversaw the production of the mesenchymal stromal cells (MSCs). PN was involved in the study design, and he and EW performed the statistical and methodological analysis. GG was the guarantor, overall study leader and was part of the design of the clinical trial and the mechanistic studies. He also performed patient evaluations.

Funding This study was funded by a grant from the Lupus Foundation of America and NIH UL1 RR029882

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by IRB00000028 Medical U South Carolina IRB 2Phase I MSCs in SLE (Pro00061632). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. We are happy to provide any additional data on reasonable request.

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