Safety and preliminary efficacy of allogeneic bone marrow-derived multipotent mesenchymal stromal cells for systemic sclerosis: a single-centre, open-label, dose-escalation, proof-of-concept, phase 1/2 study

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Summary

Background Systemic sclerosis remains an orphan life-threatening autoimmune disease. The unique immunomodulatory, proangiogenic, and antifibrotic properties of mesenchymal stromal cells provide a strong rationale for mesenchymal stromal cell-based therapy for systemic sclerosis, and treatment with mesenchymal stromal cells has shown benefits in preclinical models of this disease. The safety of allogeneic bone marrow-derived mesenchymal stromal cell administration in patients with severe systemic sclerosis has not yet been established. We aimed to test the safety and feasibility of a single intravenous injection of intrafamilial allogeneic bone marrow-derived mesenchymal stromal cells to treat severe diffuse systemic sclerosis.

Methods We did an open-label, dose-escalation, proof-of-concept, phase 1/2 study at Saint-Louis-Hospital, Paris, France. Eligible patients were aged 18–70 years with severe diffuse systemic sclerosis, who fulfilled the 2013 American College of Rheumatology and European League Against Rheumatism systemic sclerosis criteria, had a minimum modified Rodnan skin score of 15 (range 0–51), had severe lung, heart, or kidney involvement, and had inadequate response or contraindications to conventional immunosuppressive therapy or autologous haematopoietic stem cell transplantation. Patients with severe comorbidities were excluded. The first ten recipients were to receive a single intravenous infusion of 1×10^6 bone marrow-derived mesenchymal stromal cells per kg bodyweight, and the subsequent ten recipients were to be infused with a single dose of 3×10^6 bone marrow-derived mesenchymal stromal cells per kg bodyweight. The primary endpoint was immediate tolerance during infusion and within the first 10 days after infusion, measured as the occurrence of serious adverse events (grade 3 or higher) in all infused patients. Safety was assessed in all participants during the 24-month follow-up period. This study is registered with ClinicalTrials.gov, NCT02213705.

Findings Between March 24, 2014, and Jan 6, 2020, 20 cisgender individuals (13 women and seven men) with severe diffuse systemic sclerosis were enrolled. All 20 patients were included in the primary outcome analysis. No infusion-related severe adverse events and three infusion-related adverse events occurred in the first 10 days after treatment; one patient had grade 1 flushing and another patient had grade 1 nausea and grade 2 asthenia. After ten days and up to a median follow-up of 24·1 months (IQR 20·8–24·5), 36 non-treatment-related severe adverse events in 14 (70%) patients and no treatment-related adverse event were reported.

Interpretation A single infusion of allogeneic bone marrow-derived mesenchymal stromal cells was safe in patients with severe diffuse systemic sclerosis. Future placebo-controlled trials will help to definitively ascertain the efficacy of mesenchymal stromal cell-based cell therapy from various tissue sources in larger number of patients with systemic sclerosis.

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Introduction

Systemic sclerosis is a rare, chronic, systemic autoimmune disease occurring predominantly in women that substantially reduces health-related quality of life and life expectancy. Clinical manifestations derive from a pathogenic triad of early endothelial damage and vasculopathy, chronic inflammation and dysregulation of innate and adaptive immune responses, and consequent progressive fibrosis of the skin and multiple organs.[1] Disease presentation and progression vary between patients. Rapidly progressing, diffuse systemic sclerosis is the most lethal connective tissue disease and

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Research in context

Evidence before this study

Systemic sclerosis is a chronic orphan autoimmune disease that has high morbidity and the highest mortality rate among all rheumatic diseases. Systemic sclerosis is characterised by vasculopathy, dysregulation of innate and adaptive immune responses, and progressive fibrosis of the skin and internal organs. Mesenchymal stromal cells (MSCs) are multipotent cells with immunomodulatory, proangiogenic, and antifibrotic properties that have been proposed as an innovative therapeutic strategy in systemic sclerosis. Bone marrow-derived MSCs from patients with systemic sclerosis display disease-specific abnormalities, which provides a strong rationale for using allogeneic bone marrow donor sources for transplantation. We planned our study on the basis of a PubMed search, for which we used the search terms "bone marrow-derived mesenchymal stem cell transplant" AND "systemic sclerosis" OR "scleroderma" AND "1994/01/01 [Date-Publication]: 2014/03/01 [Date-Publication]". We searched for clinical trials published between Jan 1, 1994, and March 1, 2014. We identified three case reports that described seven patients with systemic sclerosis who had received bone marrow-derived MSCs obtained from different manufacturing processes. No major adverse reactions were reported and the preliminary clinical effects were encouraging. Preclinical studies testing MSCs in several animal models of systemic sclerosis have since supported the therapeutic effects of MSCs on skin and lung fibrosis.

Added value of this study

We found that a single infusion of allogeneic bone marrow-derived MSCs was safe in 20 patients with severe diffuse systemic sclerosis. Bone marrow-derived MSCs triggered disease modifying effects, with a decrease in skin fibrosis up to 1 year post-infusion, and stable forced vital capacity. Although bone marrow-derived MSCs showed manufacturing heterogeneity across donors, RNA-sequencing of the final products revealed a homogeneous transcriptomic profile. Functional evaluation of bone marrow-derived MSCs stimulated by IFNγ in vitro enabled us to identify a pattern of three co-regulated factors, including low CCL2 production, low indoleamine 2,3-dioxygenase activity, and low HLA-DR expression, which were associated with clinical non-response in patients with systemic sclerosis. In addition, non-responders had higher concentrations of circulating TGFβ than responders.

Implications of all the available evidence

To our knowledge, this is the first study to provide evidence for the use of bone marrow-derived MSCs in severe systemic sclerosis. The feasibility and safety of this therapeutic approach in patients with severe systemic sclerosis is promising, and although lung function remained stable, there was an early benefit on skin sclerosis. In order to exploit the clinical potential of MSC therapies, further randomised controlled studies with repeated MSC injections will be needed to investigate other allogeneic MSC tissue sources (eg, from adipose tissue or umbilical cords), to identify strategies that produce homogeneous batches of cells for infusion at the bedside, and to establish fully standardised in vitro safety and potency assays, which will help to identify patient subgroups who are most likely to respond to MSC treatment.

has a 5-year survival rate of 50–70% depending on the extent of organ involvement.[2] Systemic sclerosis remains an orphan disease with high unmet therapeutic need.

Not all patients benefit from treatment with immunosuppression for systemic sclerosis.[1] No biologic therapies, including those targeting T-cell co-stimulation, B cells, interleukin (IL)-6, or other specific fibrosis signalling pathways, had been approved as disease-modifying therapies for systemic sclerosis, when this trial was started. Thereafter, only the IL-6 receptor antagonist tocilizumab was approved in March, 2021, by the US Food and Drug Administration for systemic sclerosis-associated interstitial lung disease. Additionally, the antifibrotic nintedanib was recently approved for systemic sclerosis-associated interstitial lung disease. Autologous haematopoietic stem cell transplantation in patients with severe, rapidly progressing systemic sclerosis significantly improves long-term survival,[3] with regression of skin and lung fibrosis; however, the use of this treatment is contraindicated in patients with advanced visceral involvement.

Human multipotent mesenchymal stromal cells (MSCs),[4] which can be efficiently expanded from bone marrow and other tissue sources,[5,6] exhibit broad immunomodulatory, proangiogenic, and antifibrotic properties in vitro and in vivo.[7] In bleomycin-induced and hypochlorite-induced mouse models of systemic sclerosis, treatment with mouse or human MSCs reduces fibrosis in the dermis and lungs and accelerates wound healing.[7,8] These results provide a strong rationale for the use of MSCs to target the pathogenic triad of systemic sclerosis. Following the first successful treatment of refractory graft-versus-host disease, autologous and allogeneic clinical-grade MSCs were tested as therapeutics in large variety of immune-mediated diseases[4,9] and have received therapeutic market approval for Crohn's perianal fistula and graft-versus-host disease. As autologous MSCs from patients with systemic sclerosis display functional alterations,[10] the use of allogeneic MSCs is preferred for treating these patients. In addition, to fully exploit the clinical potential of MSCs, there is a need to design clinically relevant and well standardised in vitro safety and potency assays[11,12] that correlate with clinical outcomes.

We aimed to assess the safety of a single intravenous infusion of allogeneic bone marrow-derived MSCs in the treatment of severe diffuse systemic sclerosis.

Methods

Study design and participants

We did an open-label, dose-escalation, proof-of-concept, phase 1/2 study at Saint-Louis Hospital, Paris, France. Patients aged 18–70 years who fulfilled the joint 2013 American College of Rheumatology and European League Against Rheumatism systemic sclerosis criteria[13] were included if they had a minimum modified Rodnan skin score of 15 (range 0–51) and any of the following: (1) lung involvement with interstitial lung disease on chest x-ray or high-resolution CT, and a pulmonary diffusion capacity for carbon monoxide of less than 60% or a forced vital capacity (FVC) of 70% or less of the theoretical value, or alteration of FVC, or of total lung capacity of 10% or more, or both, or a 15% or greater alteration of the pulmonary diffusion capacity for carbon monoxide within the 18-month period before inclusion; (2) heart involvement with reversible congestive heart failure, ventricular or atrial rhythm disturbances, second or third degree atrioventricular block, or pericardial effusion; or (3) renal involvement with hypertension, persistent proteinuria, haematuria or casts, microangiopathic haemolytic anaemia, or new renal insufficiency plus contraindications or insufficient response to conventional immunosuppressive therapy or autologous haematopoietic stem cell transplantation.[1,3] Patients with severe comorbidities were excluded (appendix p 1).

Intrafamilial (ie, siblings or other relatives) bone marrow donors, were included if they were aged 18–65 years, had no contraindications for bone marrow donation, and, if they were female, had a negative pregnancy test and were using effective contraception. The study protocol was amended on Dec 16, 2014, to allow spouses as donors (appendix p 29). If several donors were available, the youngest person was chosen.

All patients and bone marrow donors provided written informed consent. The protocol was approved by the Ile de France 4 ethics committee. The study was done according to the Declaration of Helsinki and good clinical practice guidelines. An independent data and safety monitoring board approved the trial design and oversaw the study.

Procedures

Clinical grade allogeneic bone marrow-derived MSCs were generated by use of good manufacturing practices, according to the European Medical Agency guidelines on human cell-based medicinal products (410860/2006) and Etablissement Français du Sang standard operating procedures. Briefly, bone marrow aspirate harvested from the donor iliac crest, was directly seeded at 50 000 nucleated cells per cm² in culture chambers with Minimum Essential Medium-α (MEM-α; Macopharma, Tourcoing, France), 5% human platelet lysate (Centre de Transfusion Sanguine des Armées, Clamart, France), and 2 international units (IU)/mL heparin. MSCs were maintained in these conditions until they reached more than 50% confluence (passage 0). Bone marrow-derived MSCs were further expanded in new culture chambers, seeded at 4000 cells per cm² in MEM-α medium, 8% human platelet lysate, and 2 IU/mL heparin. When 80% confluence was reached, bone marrow-derived MSCs (final product; passage 1) were harvested and resuspended at a maximal concentration of 2×10^6 bone marrow-derived MSCs per mL in 0·9% sodium chloride containing 0·5% human albumin. The study aimed to administer 1×10^6 bone marrow-derived MSCs per kg bodyweight to the first ten patients and 3×10^6 bone marrow-derived MSCs per kg bodyweight to the subsequent ten patients if there was a low probability of excessive toxicity at 1×10^6 MSCs per kg bodyweight. If a high probability of excessive toxicity was observed at a dose of 1×10^6 MSCs per kg bodyweight, then $0\cdot5\times10^6$ bone marrow-derived MSCs per kg bodyweight were administered.

Quality controls performed on bone marrow aspirate samples, and passage 0 or passage 1 bone marrow-derived MSCs, or both, included cell count, cell viability, immunophenotyping, colony-forming unit fibroblast assay, microbial testing, karyotype analysis, and human telomerase reverse transcriptase (*hTERT*) expression analysis (full details of the methods used are in the appendix (p 4). Release specifications were: cell viability of 80% or higher; identity and purity, with 90% or more $CD73^+$ cells, 90% or more $CD90^+$ cells, 85% or more $CD105^+$ cells, and negativity (ie, ≤5% positivity) for $CD45^+$ by flow cytometry analysis (Navios, Beckman Coulter, Fullerton, USA); and negative microbial contamination testing. Out-of-specification release was accepted at the discretion of the study investigators AC and CM. Passage 0 and 1 bone marrow-derived MSC samples were cryopreserved for further analysis of the transcriptomic profile by RNA sequencing (RNA-seq) and of the immune functions of each bone marrow-derived MSC product used (full details of the methods used are in the appendix pp 4–5).

Once released, bone marrow-derived MSCs were immediately brought at room temperature to the clinical unit. To prevent infusion-related allergic reactions, 5 mg dexchlorpheniramine maleate was administered intravenously to each bone marrow-derived MSC recipient 30 min before infusion (total infusion duration was 30–45 min). Patients were continuously monitored for any adverse events during and 1 h after infusion, and they remained hospitalised for 24 h after infusion.

All patients were monitored at each clinic visit with modified Rodnan skin score assessments and health-related quality of life assessment questionnaires, which comprised the health assessment questionnaire-disability

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index with visual analogue scale, the 36-item short form general health survey, and the EQ5D. Pulmonary function tests were performed before infusion and at 3, 6 and then every 6 months during follow-up to test forced vital capacity and pulmonary diffusion capacity for carbon monoxide. As prespecified in the study protocol (appendix pp 56–57), clinical response was defined as a greater than 25% decrease in modified Rodnan skin score or a greater than 10% increase in FVC or pulmonary diffusion capacity for carbon monoxide, or both, without additional immunosuppression, except low-dose steroids.[14] Patients were classified as clinical responders or non-responders at 3 or 6 months after infusion. Biological assessments included leukocyte counts and subtype analyses, measured by flow cytometry before infusion and at 1 and 3 months after infusion. In addition, 51 plasma-soluble factors were quantified by use of the Luminex assay (BioRad, Hercules, CA, USA, and Thermo Fisher Scientific, Waltham, MA, USA) before infusion, and at 3 and 6 months after infusion (appendix pp 6–7). The presence of class I or II anti-HLA antibodies, and of donor-specific class I or II anti-HLA antibodies in recipients was ascertained before infusion, and at 1 and 3 months after infusion (appendix p 5). Donor microchimerism was evaluated before infusion and at 1 month after infusion (appendix p 5).

To characterise the immune properties of bone marrow-derived MSCs, cryopreserved passage 1 MSCs were first thawed and seeded at 4000 cells per cm² in MEM-α and 10% fetal calf serum (Biosera, Nuaille, France). MSCs were used for functional assays once they had reached 80% confluence (passage 1). MSCs were seeded at 1×10^5 cells per well on 24-well plates and stimulated for 3 days with increasing doses of interferon-gamma (IFNγ; 0, 0·8, 4, 20, 100, and 500 IU/mL; R&D Systems, Minneapolis, MN, USA). Chemokine (CXC motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 9 (CXCL9), CXCL10, IL-7, vascular endothelial growth factor (VEGF), granulocyte-colony stimulating factor (G-CSF), and IFNγ concentrations were measured in the culture supernatants (Luminex technology; Millipore, Burlington, VT, USA; appendix p 8). In all assays, IL-7, G-CSF, and IFNγ concentrations were less than the lower limit of quantification, irrespective of the amount of IFNγ stimulation; therefore, these factors were not studied further. Indoleamine 2,3-dioxygenase (IDO) activity was evaluated by liquid chromatography coupled to tandem mass spectrometry and expressed as the kynurenine to tryptophan ratio. Resting and IFNγ-stimulated bone marrow-derived MSCs were also stained with allophycocyanin-Alexa Fluor 750-conjugated anti-HLA-DR antibody (Immu-357 clone; Beckman Coulter) or isotype control and analysed by flow cytometry to measure the relative mean fluorescence intensity of HLA-DR. The mixed lymphocyte reaction was performed as described previously[15] using bone marrow-derived MSCs co-cultured at different ratios for 7 days with peripheral blood mononuclear cells pooled from 10 healthy donors and labelled with CellTrace Violet (Thermo Fisher Scientific). To calculate the percentage of division of viable T cells, peripheral blood mononuclear cells stained with 7-aminoactinomycin D-conjugated anti-CD3 and anti-CD45 antibodies were analysed with the Navios flow cytometer (Beckman coulter) and FlowJo version 10 software. The percentage of inhibition for each ratio was calculated using the following formula: $PI_{ratio\ x}=(Pdiv_{control}-Pdiv_{ratio\ x})/Pdiv_{control}$, where PI is the percentage of inhibition and Pdiv is the percentage of division. The area under the curve for each batch of bone marrow-derived MSCs was calculated using the trapezoidal rule.

All primary and secondary endpoint data, as well as bone marrow-derived MSC production process data, were entered into an electronic database. Data quality was monitored by dedicated staff independent of the investigator site, with 100% source data verification for all patients. A data and safety monitoring board reviewed all severe adverse event data and biological data, if necessary, after every four patients were treated.

Outcomes

The primary objective was the safety of allogeneic bone marrow-derived MSC infusion. The primary endpoint was immediate tolerance during infusion and within the first 10 days after bone marrow-derived MSC infusion, specifically the occurrence of severe adverse events (ie, grade 3 or higher) according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. Treatment-related and non-treatment related adverse events were declared by the investigator, adjudicated by the sponsor, and reviewed by the data safety monitoring board (appendix pp 73–75).

Secondary objectives included the feasibility of bone marrow-derived MSC preparation and infusion, tolerability at all follow-up visits, descriptive analysis of the observed clinical response, treatment efficacy, and a mechanistic analysis of clinical response. Secondary endpoints were adequacy of bone marrow-derived MSC production, the occurrence of any adverse events during the 24-month follow-up period, routine clinical and biological characteristics at 1 month and once every 3 months during follow-up, clinical responses, progression-free survival and overall survival at 12 months, recipient immune response measures (lymphocyte phenotyping, lymphocyte subset counts, alloimmunisation (detection of donor-specific anti-HLA antibodies), and cytokine plasma concentrations before infusion, and at 1 and 3 months after infusion. Progression-free survival, was defined as the time (in days) from the day of inclusion until the occurrence of one of the following changes compared to the initial assessment, documented and re-evaluated at two successive examinations 3 months apart: a greater than 10% decrease in FVC or a 15% reduction in pulmonary diffusion capacity for carbon monoxide (compared with the theoretical

value), or both; a greater than 15% decrease in left ventricular ejection fraction on isotopic measurement of the ejection fraction; a 15% decrease in the patient's weight; a 30% reduction in creatinine clearance; a 30% increase in the modified Rodnan skin score; a 0·5 increase in the health-related quality of life health assessment questionnaire-disability index score. Overall survival was defined as the time period between enrolment and death from any cause.

We did a post-hoc analysis of the association between the biological features of infused recipients and functional characteristics of bone marrow-derived MSCs in patients with and without a clinical response at 3 or 6 months.

Statistical analysis

Continuous variables are summarised as medians (with IQRs) and categorical variables as numbers (with percentages). Primary endpoint analyses were done sequentially for the first ten patients included at a target dose of 1×10^6 bone marrow-derived MSCs per kg bodyweight, then for the subsequent ten patients included at a target dose of 3×10^6 bone marrow-derived MSCs per kg bodyweight. Primary and secondary endpoints were assessed in all infused participants. The probability of intolerance was estimated with Bayesian inference. The Bayesian approach considers the rate of intolerance (π) as a random variable with a previous density, which is updated with the observations to a so-called posterior density. The mean of the posterior π density is given with its 95% credibility interval (CrI). Analysis of clinical evolutive secondary endpoints was descriptive only. Medians (IQRs) were estimated and presented as boxplots with whiskers extended to the most extreme datapoint, which is no more than 1·5 times the IQR from the box. All time-point differences were analysed by use of Friedman tests. When a significant difference was detected, pair-wise comparisons of the differences were done ad-hoc using paired Wilcoxon signed rank tests. Co-regulated variables identified in the functional characteristics of bone marrow-derived MSCs were evaluated in a post-hoc analysis with Pearson correlation coefficients after normalisation, and expressed with a heatmap. The Mann-Whitney test was used for independent sample comparisons.

All tests had a two-sided α level of 0·05. Statistical analyses were done using SAS version 9.4, R version 4.0.3, and GraphPad Prism version 9.1.1. Correlation analyses and heatmaps were done with corrplot version 0.84, pheatmap version 1.0.12, and amap version 0·8–18 R packages.

The study is registered with ClinicalTrials.gov, NCT02213705.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

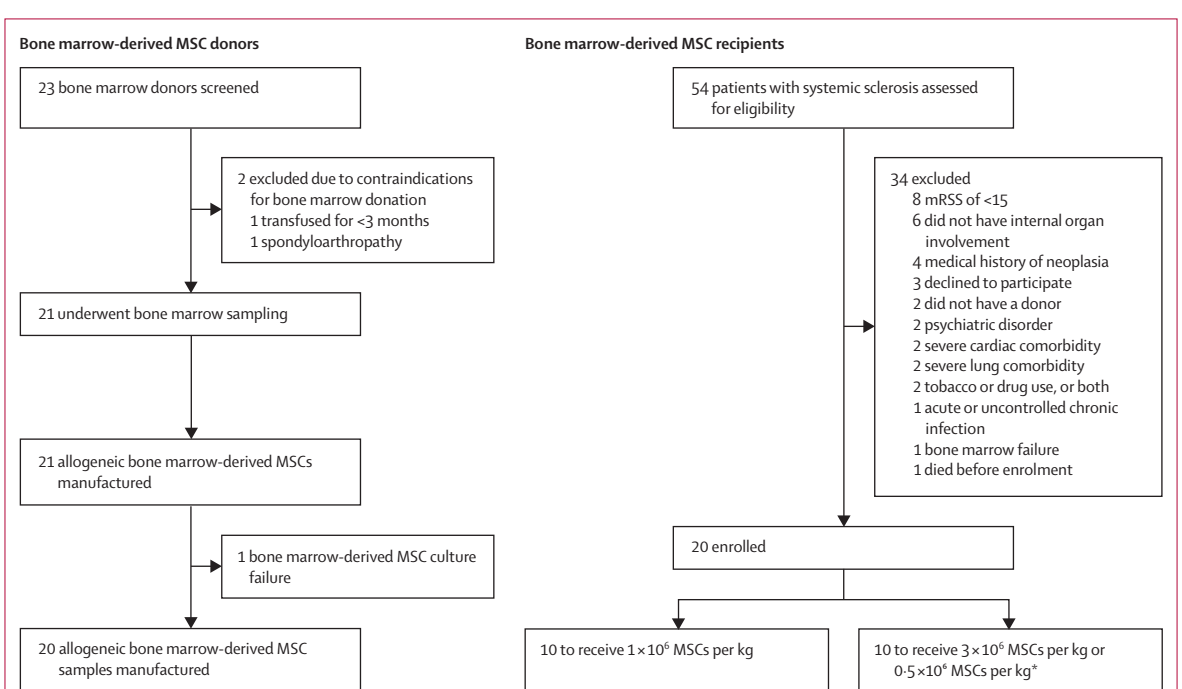

Figure 1: **Study profile**

mRSS=modified Rodnan skin score. MSCs=mesenchymal stromal cells. *Patients were expected to be given this dose depending on toxicity results in patients who received 1×10^6 MSCs per kg bodyweight.

	Participants (n=20)
Age, years	47 (36–57)
Gender	
Female	13 (65%)
Male	7 (35%)
Disease duration, years	6·9 (4·6–10·0)
Diffuse systemic sclerosis	20 (100%)
Geographical region	
Sub-Saharan Africa	2 (10%)
Europe	14 (70%)
North Africa	2 (10%)
Other	2 (10%)
Steroid treatment	7 (35%)
Daily dose at inclusion, mg/day	5 (5–7)
Previous immunosuppressive drugs	
Cyclophosphamide	11 (55%)
Azathioprine	1 (5%)
Penicillamine	2 (10%)
Methotrexate (oral)	5 (25%)
Methotrexate (subcutaneous)	2 (10%)
Mycophenolate mofetil	16 (80%)
Autologous haematopoietic stem-cell transplant	6 (30%)
Rituximab	1 (5%)
Tocilizumab	1 (5%)
Immunoglobulins	1 (5%)
Duration of immunosuppressive drug exposure, months	
Cyclophosphamide	9 (9 [45%])*
Azathioprine	72
Penicillamine	142
Methotrexate (oral)	7 (4 [20%])*
Methotrexate (subcutaneous)	29
Mycophenolate mofetil	20 (14 [70%])*
Autologous haematopoietic stem cell transplant	NA
Rituximab	1
Tocilizumab	6
Immunoglobulins	9
Cutaneous and juxta-articular involvement	
Digital ulcers	9 (45%)
Raynaud's phenomenon	16 (80%)
Modified Rodnan skin score†	23 (21–29)
Tendon friction rub	16/18 (89%)
Cardiovascular function	
Systolic blood pressure, mmHg	116 (104–123)
Diastolic blood pressure, mmHg	70 (63–77)
Heart rate, beats per min	75 (67–82)
Abnormal electrocardiogram‡	11 (55%)
Left ventricular ejection fraction (by echocardiography)	65% (53–68)
Systolic pulmonary artery pressure (by echocardiography), mmHg	30 (25–35)
Pericardial effusion (by echocardiography)	1 (5%)

(Table 1 continues on next page)

Results

Between March 24, 2014, and Jan 6, 2020, 54 patients with severe systemic sclerosis (including 29 women and 25 men) and 23 bone marrow donors (including 13 women and ten men) were screened for eligibility, and 20 cisgender patients with diffuse systemic sclerosis requiring 21 cisgender bone marrow donors were included in the study (figure 1). The baseline characteristics of participants are presented in table 1. All 20 patients received a single injection of allogeneic bone marrow-derived MSCs (median dose 1 [IQR 1–3]×10⁶ MSCs per kg bodyweight) and were included in all outcome analyses. No participant received 0·5×10⁶ bone marrow-derived MSCs per kg bodyweight. During infusion or up to day 10, no severe adverse events occurred in the first ten patients, allowing for enrolment of the following 10 patients at a dose of 3×10⁶ bone marrow-derived MSCs per kg bodyweight. Two (10%) patients had transient infusion-related adverse events; one had grade 1 flushing during infusion and one had grade 1 nausea and grade 2 asthenia within the first week after infusion. After a median follow-up of 24·1 (IQR 20·8–24·5) months, no treatment-related severe adverse events, 36 non-treatment-related severe adverse events (in 14 patients), and 193 adverse events (in 19 patients) were reported (table 2; appendix pp 9–12). The mean posterior probability of treatment-related severe adverse events was estimated as 1·0% (95% CrI 0·0–7·1).

After quality control of the final bone marrow-derived MSC products had been completed (appendix p 13), five batches were released out of specification with a lower than expected target dose or cell viability, or both. At least 1×10⁶ bone marrow-derived MSCs per kg bodyweight were delivered to 18 (90%) of 20 patients. Initial bone marrow products showed large variability in cell counts and viability, population doubling times, and numbers of colony-forming units during the bone marrow-derived MSC expansion process at passages 0 and 1 (appendix pp 14–15).

After bone marrow-derived MSC infusion, an early improvement in median modified Rodnan skin score was observed, which persisted over the 12-month follow-up period (figure 2A). FVC, pulmonary diffusion capacity for carbon monoxide, and heart or renal function parameters appeared stable over the 12-month follow-up period (figure 2B; appendix p 16). The health-related quality of life health assessment questionnaire-disability index [baseline score 1·50 (IQR 0·72–1·63)] and the 36-item short form general health survey score [baseline score 44 (31–53)] remained stable during the 12-month follow-up period. Considering the participant's clinical response status[14] at 3 or 6 months after bone marrow-derived MSC infusion, 15 (75%) of 20 patients were classified as responders and five (25%) as non-responders (estimated probability of response 0·74 (95% CrI 0·54–0·90)]. The probability of a clinical response was 0·77 (95% CrI 0·50–0·96]) in patients who received 1×10⁶ allogeneic bone marrow-derived MSCs per kg bodyweight and 0·68 (0·39–0·91) in those who received 3×10⁶ allogeneic bone marrow-derived MSCs per kg bodyweight. No deaths were reported during the

24-month follow-up period. Progression-free survival at 12 months was 75% (95% CrI 58–97).

Total lymphocyte, monocyte, B-cell, and T-cell counts were not modified by bone marrow-derived MSC infusion (appendix p 20). $CD4^+$ T-cell counts remained stable between baseline and 3 months after infusion, whereas $CD8^+$ T-cell counts increased at 1 month (p=0·025), and natural killer cell counts increased at 1 month (p=0·031) and 3 months (p=0·0080) after allogeneic bone marrow-derived MSC infusion. No association between immune cell subset counts before treatment and clinical response was observed (appendix p 21).

Before bone marrow-derived MSC infusion, three (15%) of 20 patients had donor-specific class I or II anti-HLA antibodies, which disappeared at 1 month after infusion (n=1) or remained positive until 3 months after infusion (n=2). Two (10%) patients had de novo donor-specific class I anti-HLA antibodies at 1 month after infusion, and they remained positive for these antibodies 3 months after infusion (appendix p 17). 16 (80%) patients were informative for qPCR analysis of non-shared donor HLA. Low levels of chimerism were detected in three (15%) patients (appendix p 22), and were verified not to be of donor origin, but were instead from naturally-acquired microchimerism before transplantation (appendix p 22), The concentration of the 39 quantifiable plasma-soluble circulating factors in patients with systemic sclerosis were not significantly modified between infusion and at 3 or 6 months after infusion (data not shown). All patients in our study had elevated blood plasma concentrations of TNF (44·6 pg/mL [IQR 36·3–57·3]) and IFNγ (17·2 pg/mL [12·5–21·0]) before bone marrow-derived MSC infusion. Conversely, plasma concentrations of TGFβ were significantly higher in clinical non-responders than in clinical responders (p=0·018; figure 3A), whereas a module of co-regulated inflammatory factors, including IFNγ and TNF, and other MSC-activation factors, was identified and found not to be associated with treatment response (appendix p 23).

Karyotype analysis revealed chromosomal abnormalities in three (15%) of 20 final bone marrow-derived MSC products (appendix p 19), all of which disappeared during subsequent long-term cultures. These cultures were used to assess population doubling time, perform karyotype analysis, and analyse *hTERT* expression at each passage until bone marrow-derived MSCs entered senescence (appendix p 19). Bone marrow-derived MSCs progressively reached growth arrest, did not express *hTERT*, and showed no evidence of transformation in vitro.

The final bone marrow-derived MSC product gene expression profiles, analysed by RNA-seq (in 17 batches), showed that the expansion protocol yielded an overall homogenous cell product, with cells expressing classic MSC genetic markers (*THY1*, *ENG*, *STRO1*, and *VCAM1*) and being free of haematopoietic cells (appendix p 24). Conversely, the immunosuppressive properties of final bone marrow-derived MSC products, as assessed by mixed lymphocyte reaction, revealed large heterogeneity between the 13 tested bone marrow-derived MSC batches (median area under the curve 0·10 [IQR 0·05–0·12]; coefficient of variation 46·8%). Neither bone marrow-derived MSC-mediated inhibition of T-cell proliferation, karyotypic abnormalities, nor patient clinical outcome were associated with differences in the transcriptional profiles of bone marrow-derived MSCs. Following exposure of bone marrow-derived MSC batches to increasing doses of IFNγ, CCL2 and VEGF were constitutively expressed, whereas CXCL9 and CXCL10 expression and IDO activity were induced, and HLA-DR was variably upregulated by IFNγ (appendix p 25). In agreement, we further analysed the

	Participants (n=20)
(Continued from previous page)	
Pulmonary function	
Rales	15 (75%)
Interstitial lung disease§	20 (100%)
Abnormal chest x-ray	17/19 (89%)
Abnormal high-resolution CT	19/19 (100%)
Partial pressure of oxygen at room air, mmHg	100 (92–113; 19 [95%])*
Pulmonary function tests	
Percent predicted vital capacity	70·5% (63·0–78·8; 18 [90%])*
Percent predicted total lung capacity	77% (72·5–95·5; 19 [95%])*
Percent predicted forced vital capacity	69% (66·5–80·5; 19 [95%])*
Percent predicted DLCO	39·8% (34·7–50; 19 [95%])*
Gastrointestinal tract function	
Serum albumin, g/L	39·5 (36·8–41·3)
Body-mass index, kg/m²¶	23·5 (18·7–24·5)
Parenteral nutrition	1 (5%)
Renal function	
Serum creatinine <120 µmol/L	54·5 (44·8–73·3)
Proteinuria	5 (25%)
History of renal crisis	1 (5%)
Biological and immunological values	
Haemoglobin concentration, g/dL	11·9 (11·2–12·2)
Leukocyte count, 10^9 cells per L	5·39 (4·49–6·59)
Neutrophil count, 10^9 cells per L	3·30 (2·61–4·23)
Lymphocyte count, 10^9 cells per L	1·26 (1·09–1·61)
Platelet count, 10^9 platelets per L	227 (180–274)
C-reactive protein concentration <6 mg/L	2 (2–6)
Antinuclear antibody positive	19 (95%)
Anti-Scl70 antibody positive	16 (80%)
Anti-centromere antibody positive	0 (0%)
Anti-RNP antibody positive	2 (10%)
Anti-RNA polymerase III antibody positive	2 (10%)
Performance status	
0	1 (5%)
1	15 (75%)
2	3 (15%)
3	1 (5%)

(Table 1 continues on next page)

	Participants (n=20)
(Continued from previous page)	
Quality of life	
HAQ-DI‖	1·50 (0·72–1·63)
Visual analogue scale HAQ score**	21·3 (4·4–40·8; 18 [90%])*
EQ5D	
Index-based utility score††	0·68 (0·48–0·77; 18 [90%])*
Visual analogue scale EQ5D score‡‡	60·0 (47·5–70·0; 19 [95%])*
SF36 global§§	44 (31–53)
SF36 physical component§§	29 (22–47)
SF36 mental component§§	56 (40–69)

Data are median (IQR), n (%), n/N (%), or median (IQR; n [%]), unless otherwise indicated. Baseline characteristics were recorded at the time of hospitalisation for infusion with allogeneic bone marrow-derived mesenchymal stromal cells. In case of missing values, the most recent anterior value recorded since eligibility was considered. DLCO=pulmonary diffusion capacity for carbon monoxide. HAQ-DI=health assessment questionnaire-disability index. NA=not available. SF36=36-item short form general health survey. *n (%) or n [%] indicates the number and proportion of patients with available data for that variable. †Scores can range from 0 to 51, with higher scores indicating more severe skin thickening. ‡Defined as the presence of atrial or ventricular rhythm disturbances, such as recurrent episodes of atrial fibrillation or flutter, recurrent atrial paroxysmal tachycardia or ventricular tachycardia, second-degree or third-degree atrioventricular block, or diffuse microvoltage or repolarisation abnormalities related to pericardial effusion; non-scleroderma-related causes were excluded. §Defined as the presence of bronchiolar involvement, or ground glass opacification or fibrosis on chest x-rays or the high-resolution CT scan, or both; other causes of clinically relevant obstructive disease and emphysema were excluded. ¶Calculated as weight in kg divided by height (in m) squared. ‖Scores can range from 1 to 3, with lower scores indicating less disability. **Used to evaluate pain intensity due to systemic sclerosis during the previous week before evaluation, with scores ranging from 0 (no pain) to 100 (very severe pain). ††Typically interpreted along a continuum, in which 1 represents best possible health and 0 represents death. ‡‡Evaluates health status, with scores ranging from 0 (worst imaginable health status) to 100 (best imaginable health status). §§Scores can range from 0 to 100, with higher scores indicating better health status.

Table 1: **Baseline characteristics of patients with severe systemic sclerosis**

constitutive CCL2 and VEGF expression levels, the CXCL9 and CXCL10 expression levels measured with the maximal IFNγ dose (500 IU/mL), the IDO activity obtained at the previously identified optimal dose of 20 IU/mL IFNγ,[11] and the HLA-DR expression at the half-maximal effective concentrations of IFNγ. Pearson correlation analysis identified two groups of co-regulated factors; one included IDO activity, CCL2 concentrations, and HLA-DR expression, and the other included CXCL9, CXCL10, and VEGF concentrations (figure 3B). We then evaluated whether these patterns of co-regulated factors in bone marrow-derived MSCs were related to clinical response in recipients. Although none of the tested factors were able to discriminate clinical responders from clinical non-responders on their own (figure 3C), clustering of IDO activity, CCL2 concentration, and HLA-DR expression revealed two groups; one showing higher levels of these factors, which included only clinical responders, and the other showing lower expression levels of these factors, which included the four non-responders (figure 3D).

Discussion

This dose-escalation study, involving 20 patients with diffuse systemic sclerosis and severe skin and lung involvement at enrolment, shows the safety of a single infusion of $1–3\times10^6$ allogenic bone marrow-derived MSCs per kg bodyweight with at least 1 year of follow-up. The results also highlight the important variability in bone marrow sampling and bone marrow-derived MSC proliferation capacity across donors, which could hamper efforts to obtain reproducible batches with prespecified doses per bodyweight despite using well-established standardised techniques for bone marrow-derived MSC production.[11] Nonetheless, we observed regression of skin sclerosis early after infusion, and stable pulmonary function until 1 year post infusion.

The probability of a clinical response was similar between participants who received 1×10^6 bone marrow-derived MSCs per kg bodyweight and those who received 3×10^6 bone marrow-derived MSCs per kg bodyweight, indicating that minimal MSC doses could be effective with a wide dose-response therapeutic window, as has been reported in other MSC trials[16] and in mouse models of systemic sclerosis.[8] Although one might hypothesise that patients with diffuse systemic sclerosis could improve spontaneously, these favourable results prompted us to assess the mechanisms of action of bone marrow-derived MSCs. We did a comprehensive analysis of the initial immune and inflammatory status of recipients at the time of transplantation, together with the immune properties of allogeneic bone marrow-derived MSCs, to model their combined potential interaction with clinical response and to identify predictive biomarkers of therapeutic activity.

With safety as the primary endpoint, we designed an adaptive Bayesian non-randomised phase 1/2 study, with quantitative interim monitoring for unacceptable toxicity, to safely increase the injected dose of bone marrow-derived MSCs. Eligibility and endpoint criteria were in accordance with the Outcome Measures in Rheumatology Clinical Trials filters for systemic sclerosis,[17] with patients being their own control for preliminary evaluation of efficacy outcomes.

The primary safety endpoint was chosen at 10 days to assess early toxicity of bone marrow-derived MSC infusion, considering the documented short half-life of circulating MSCs after infusion.[18] In agreement with an updated meta-analysis,[9] which evaluated the risk–benefit profile of MSC infusion for various disorders, we found no evidence of immediate or longer-term (ie, up to 24 months) treatment-related severe adverse events, including infection, thromboembolism, or malignancy. When considering the prior distribution derived from the data of Thompson and colleagues,[9] and the absence of any treatment-related severe adverse events in our study, the probability of treatment-related adverse events is less than 1% (ie, lower than our initial estimate). Although clinical-grade MSCs can acquire random and spontaneous genetic aberrations during in vitro expansion, as observed in the three last bone marrow-derived MSC products in our study, it has now been extensively shown that these chromosomal alterations related to replicative senescence do not confer selective growth advantages nor an increased risk of transformation.[19,20] Transformation of MSCs has never been reported in humans after almost 20 years of clinical use.[9]

	Number of patients with severe adverse event		Number of patients with non-severe adverse events	Number of severe adverse events		Number of non-severe adverse events
	Day 0–10	Day 11–month 24		Day 0–10	Day 11–month 24	
Total	0	14	19	0	36	193
Total infusion-related events	0	0	2	0	0	3*
Cardiac disorders	0	6	8	0	13	18
Aortic valve disease	0	0	1	0	0	1
Arterial hypertension	0	1	1	0	1	1
Complete atrioventricular block	0	1	0	0	1	0
Auricular arrhythmia	0	0	0	0	0	0
Cardiac arrest	0	1	0	0	1	0
Limb oedema	0	0	1	0	0	1
Mitral valve disease	0	0	5	0	0	5
Palpitations	0	0	2	0	0	2
Right ventricular dysfunction	0	0	2	0	0	3
Sick sinus syndrome	0	1	0	0	1	0
Sinus tachycardia	0	1	0	0	1	0
Tricuspid valve disease	0	0	3	0	0	3
Ventricular arrhythmia	0	1	1	0	2	1
Ventricular tachycardia	0	1	0	0	1	0
Other	0	3	1	0	5	1
Ear and labyrinth disorders	0	0	1	0	0	1
Endocrine disorders	0	0	1	0	0	1
Eye disorders	0	1	4	0	1	4
Gastrointestinal disorders	0	4	13	0	5	25
Anorexia	0	1	0	0	1	0
Chronic intestinal pseudo-obstruction	0	3	2	0	3	2
Diarrhoea	0	0	3	0	0	3
Faecal incontinence	0	0	2	0	0	2
Nausea	0	0	2	0	0	2
Periodontal disease	0	0	2	0	0	3
Vomiting	0	0	2	0	0	4
Other	0	1	7	0	1	9
General disorders and administration site conditions	0	0	8	0	0	12
Hepatobiliary disorders	0	0	1	0	0	1
Infections and infestations	0	4	10	0	7	23
Kidney infection	0	1	0	0	1	0
Lung infection	0	0	2	0	0	3
Prostate infection	0	1	1	0	2	1
Skin infection	0	0	6	0	0	8
Viral lung infection	0	2	0	0	3	0
Urinary infection	0	1	2	0	1	2
Other	0	0	5	0	0	9
Injury, poisoning, and procedural complications	0	1	0	0	1	0
Laboratory investigations	0	1	9	0	1	11
Anaemia	0	1	5	0	1	6
Other	0	0	5	0	0	5
Metabolism and nutrition disorders	0	0	4	0	0	4
Musculoskeletal and connective tissue disorders	0	0	12	0	0	23
Arthralgia	0	0	7	0	0	9
Myalgia	0	0	4	0	0	4
Other	0	0	8	0	0	10

(Table 2 continues on next page)

	Number of patients with severe adverse event		Number of patients with non-severe adverse events	Number of severe adverse events		Number of non-severe adverse events
	Day 0–10	Day 11–month 24		Day 0–10	Day 11–month 24	
(Continued from previous page)						
Benign, malignant, or unspecified neoplasm	0	1	2	0	1	2
Nervous system disorders	0	0	8	0	0	12
Anxiety	0	0	3	0	0	3
Headache	0	0	2	0	0	2
Others	0	0	5	0	0	7
Renal and urinary disorders	0	1	1	0	1	2
Erectile dysfunction	0	0	1	0	0	2
Respiratory, thoracic, and mediastinal disorders	0	1	10	0	1	24
Cough	0	0	2	0	0	3
Dyspnoea	0	0	8	0	0	10
Pleural effusion	0	1	0	0	1	0
Pulmonary hypertension	0	0	2	0	0	2
Voice alteration	0	0	2	0	0	3
Other	0	0	5	0	0	6
Skin and subcutaneous tissue disorders	0	4	8	0	4	22
Skin ulceration	0	4	5	0	4	12
Other	0	0	6	0	0	10
Vascular disorders	0	1	5	0	1	6
Flushing	0	0	1	0	0	1
Thrombophlebitis	0	0	2	0	0	2
Other	0	1	2	0	1	3

Adverse events were graded according to Common Terminology Criteria for Adverse Events version 5.0. Day 0 was the day of allogeneic bone marrow-derived mesenchymal stromal cell infusion. *Included one patient with flushing during infusion, and one with nausea and asthenia.

Table 2: **Adverse events reported from time of allogeneic bone marrow-mesenchymal stromal cell infusion until 12 months in all treated patients with severe diffuse systemic sclerosis**

There is a strong rationale for using allogeneic rather than autologous MSCs to treat systemic sclerosis.[7] Culture-adapted MSCs from patients with systemic sclerosis show functional abnormalities, including abnormal proliferation rates, aberrant metabolic, migration, and differentiation profiles, and abnormal fibrotic activity.[10] However, the use of allogeneic MSCs can lead to alloimmunisation. MSCs are not immune-privileged,[21] and induction of anti-HLA class I antibodies with as yet unknown clinical consequences was reported after infusion of allogeneic MSCs derived from bone marrow, adipose tissue, and umbilical cord.[22] Although both a single dose of allogeneic or syngeneic bone marrow-derived MSCs improve colitis outcomes in mice, only syngeneic MSCs allow a sustained treatment response with repeated doses for relapsing colitis. Following treatment of perianal fistulas with a single local injection of allogeneic adipose-derived stromal cells in patients with Crohn's disease, 17 (32%) of 53 patients developed de novo donor-specific class I anti-HLA antibodies at concentrations that were insufficient to trigger antibody-dependent cytotoxicity and death of the adipose-derived stromal cells in vitro, or to affect the clinical efficacy of these cells.[22] In our study, no direct effect of donor-specific class I or II anti-HLA antibodies on clinical response to bone marrow-derived MSC infusion occurred, regardless of the antibody concentrations, as MSCs appear to be rapidly eliminated in vivo by immune-mediated apoptosis followed by efferocytosis.[23] Consistent with these results, no detectable MSC-related donor microchimerism was found in our study.[24] Although phagocytosis by the recipient's macrophages has been proposed as a key mechanism for MSC-mediated immunological effects in treating graft versus host disease,[23] transient engraftment of living, metabolically active MSCs in vivo is essential in several clinical settings, suggesting that production of immunosuppressive factors by so-called fit MSCs is important for their clinical activity.[25]

MSCs are thought to release a complex set of immunosuppressive molecules in the presence of by inflammatory stimuli.[26] Two levels of heterogeneity should be considered in clinical trials of MSCs when searching for biomarkers of clinical activity; the inflammatory status of the recipient and the intrinsic functional capacities of the donor MSCs. Therefore,

we first quantified the concentrations of circulating inflammatory cytokines, growth factors, chemokines, and biomarkers associated with the pathogenesis of systemic sclerosis.[27] We identified a subset of patients with an exacerbated inflammatory profile, which was not a predictor of clinical response. Severe systemic sclerosis is characterised by high circulating concentrations of TNF and IFNγ, and all patients in our study displayed elevated blood plasma concentrations of these factors before bone marrow-derived MSC infusion. TGFβ concentrations were significantly elevated in clinical non-responders compared with clinical responders. TGFβ, a pivotal driver of fibrosis and a putative therapeutic target in systemic sclerosis, synergises with other profibrotic factors, including IL-6 and platelet-derived growth factor, which all belong to the same cluster of co-regulated factors identified in our study.[28] Whether and how TGFβ or other related profibrotic factors might counteract MSC activity in this clinical setting remains to be explored.

Despite bone marrow donor-related heterogeneity and MSC manufacturing differences, all clinical-grade allogeneic bone marrow-derived MSC batches used in our study comprised homogeneous cell products, as documented by RNA-seq analysis. To explore the functional heterogeneity of different bone marrow-derived MSC batches, we quantified the expression of soluble and membrane factors related to the capacity of MSCs to inhibit T cells in vitro.[11,29] Based on this strategy, we found that low IDO activity, low CCL2 production, and low HLA-DR expression following IFNγ stimulation were associated with a clinical non-response. The plurality of MSC effector pathways could explain why several factors were associated in this analysis. IDO activity is required for the T-cell inhibitory functions of human MSCs in vitro.[11] CCL2 has progressively emerged as a key MSC-derived factor in experimental models of autoimmune neuroinflammation and sepsis via the recruitment and conversion of CCR2-expressing myeloid cells.[30,31] Additional data from a large series of patients treated for systemic sclerosis will be required to determine the reliability of the multifactor in-vitro assay used in our study in determining MSC potency.

There are some limitations with regards to the design of this open-label study. First, due to the wide variation in the rate of systemic sclerosis progression between patients, it is difficult to ascertain whether the observed changes in modified Rodnan skin score and stabilisation of pulmonary function were associated with the intervention rather than the natural disease history, given that there was no control group. However, safety was our primary aim, and a placebo-controlled design was not mandatory. Second, as this study included patients with established systemic sclerosis (average disease duration >5 years), it is unclear whether the intervention would be safe and effective in patients with an earlier disease stage; the stage at which patients often experience the most rapid progression of skin and lung disease secondary to systemic sclerosis. Third, because additional immunosuppressant therapies were allowed in patients with disease progression, potential differences between the two treatment doses were assessed only at 3-months and 6-months of follow-up. Therefore, we cannot draw definitive conclusions about treatment efficacy. Finally, the type of intervention studied might not be a feasible treatment option for patients who are not followed up at academic centres.

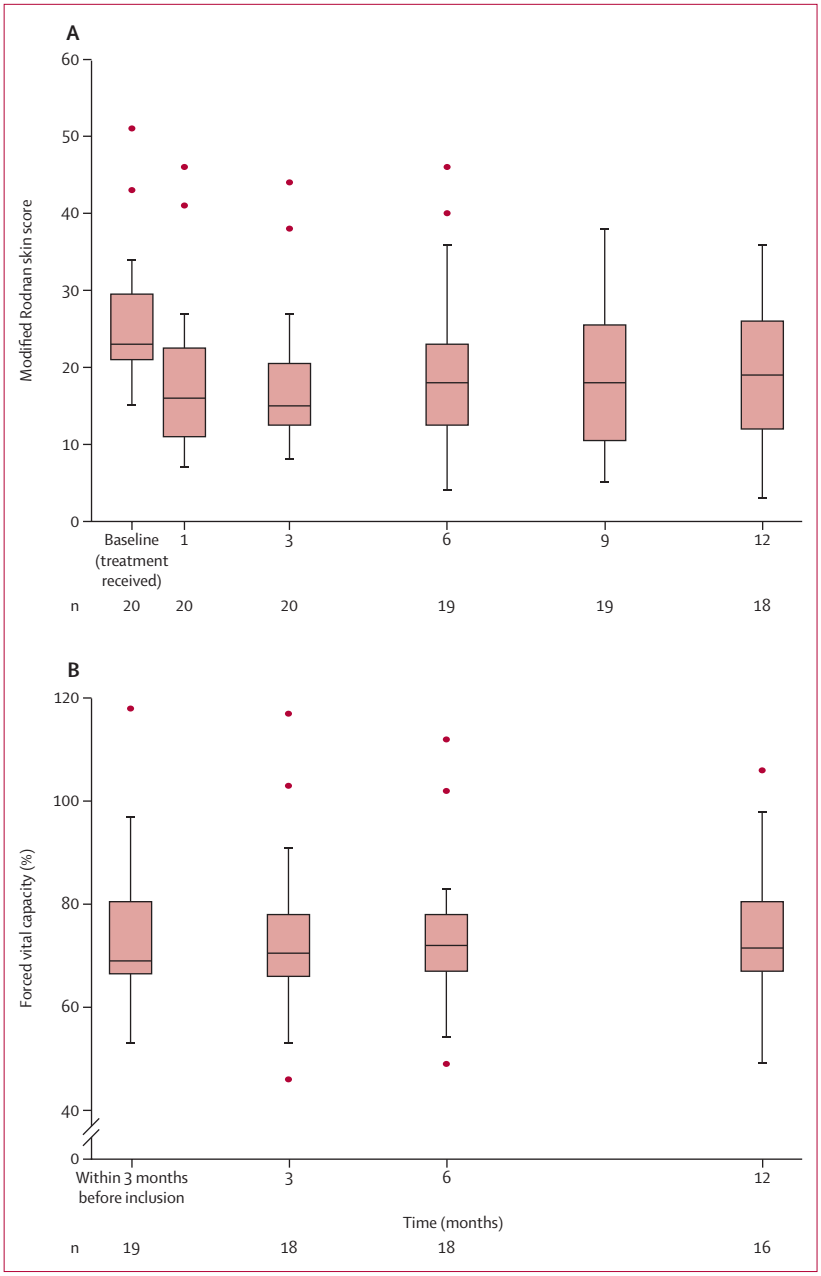

Figure 2: **Changes in modified Rodnan skin score (A) and forced vital capacity (B) from baseline to 12 months after allogeneic bone marrow-mesenchymal stromal cell infusion in patients with severe systemic sclerosis**
Filled circles indicate datapoints outside the boundary of the whiskers. n=number of patients assessed at each timepoint.

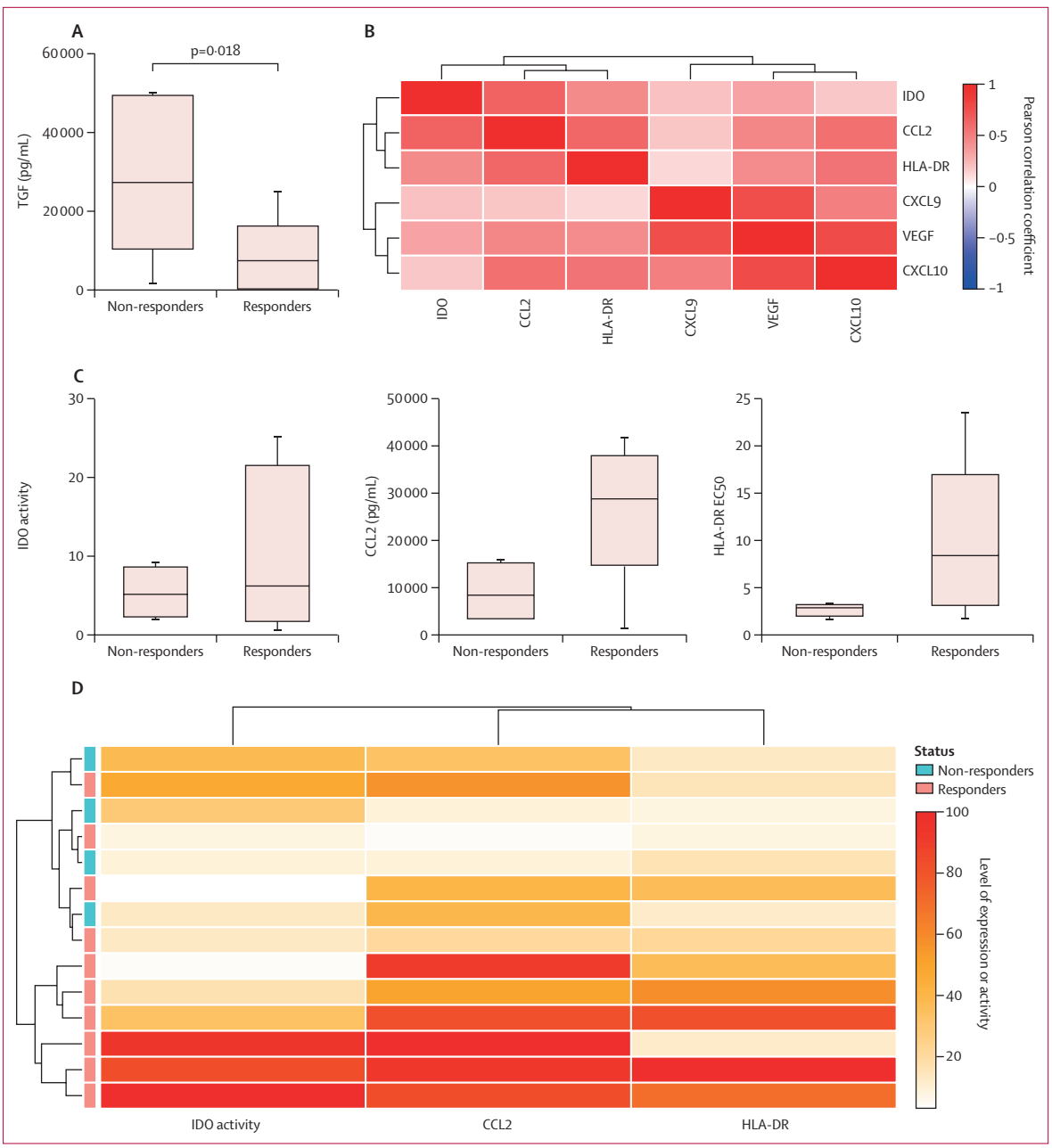

Figure 3: **TGFβ expression and immune properties of allogeneic bone marrow-derived MSCs as indicators of clinical efficacy in patients with severe systemic sclerosis**

(A) TGFβ was quantified with a Luminex assay in peripheral blood at baseline (day 0). Data obtained in clinical responders and non-responders to allogeneic bone marrow-derived MSC treatment were compared with a Mann-Whitney test. Each batch of bone marrow-derived MSCs (n=14) was stimulated for 3 days with increasing doses of IFNγ before culture supernatants and cells were collected. IDO activity was quantified by liquid chromatography coupled to tandem mass spectrometry, soluble factor concentrations by Luminex assay, and HLA-DR expression by flow cytometry. (B) A Pearson correlation heatmap showing co-regulated patterns between the most relevant immunological factors (after normalisation) . (C) IDO activity, CCL2 production, and HLA-DR expression in clinical responders and non-responders. (D) Heatmap showing normalised values for CCL2 production, HLA-DR expression, and IDO activity, highlighting expression patterns in clinical responders and non-responders. Relative expression or activity levels were normalised by attributing the value of 100 to the maximum value. EC50=half-maximal effective concentration. IDO=indoleamine 2,3-dioxygenase. MSC=mesenchymal stromal cell.

In conclusion, a single intravenous infusion of allogeneic bone marrow-derived MSCs, at a dose of up to 3×10^6 MSCs per kg bodyweight, was shown to be safe in patients with severe systemic sclerosis, with promising results suggesting that this treatment promotes skin improvement. Future double-blind, randomised, placebo-controlled trials assessing repeated MSC infusions in a larger number of patients,

with careful longitudinal monitoring of immune responses, are required to definitively ascertain the therapeutic efficacy of MSC-based cell therapy in systemic sclerosis. MSC batch selection, taking into account previous or secondary (ie, after bone marrow-derived MSC infusion) alloimmunisation, and MSC quality assessment using quantitative, reproducible assays that analyse their capacity to interact with T cells and myeloid cells, is recommended to be included in such approaches. Given the importance of the tissue of origin on the functional properties of MSCs,[32] the use of adipose-derived stromal cells (NCT03211793 and NCT04356755) or umbilical cord-derived MSCs (NCT04356287), which exhibit an immune profile consistent with stronger inhibition of the immune response or lower immunogenicity than bone marrow-derived MSCs, should be considered.

Contributors

DF, MR-R, LS, and KT conceptualised the study and outlined the research goals. DF, MR-R, IC, DL, VDK, NCL, LS, AC, and KT designed the clinical trial or biological experiments. DF, IC, LS, and KT provided oversight, leadership, and mentorship. DF and KT were involved in funding acquisition. DF, PL, CC, LS, AC, and KT managed and coordinated research activity planning and execution. DF, SL, PL, DL, CC, GP, ATJM, EC, EH, TM, VDK, NCL, DM, CM, HCW, LS, AC, and KT conducted research activities and performed experiments, including enrolling patients, collecting bone marrow samples, the production of MSCs, and collecting data. DF, SL, MR-R, PL, DL, CC, VDK, NCL, CM, H-CW, LS, AC, and KT provided oversight, verification, and validation of the data or were responsible for data curation, or both. DF, SL, MR-R, DL, VDK, NCL, H-CW, CM, AC, and KT analysed the clinical and biological data. MR-R and DL were responsible for the design and development of programming software for the statistical analysis. DF, SL, MR-R, DL, VDK, NCL, AC, and KT wrote the original drafts of this manuscript. DF, SL, MR-R, PL, DL, CC, VDK, NCL, H-CW, AC, and KT prepared the figures and tables for publication. DF, MR-R, DL, GP, ATJM, EC, EH, TM, CM, LS, AC, and KT enrolled patients and provided study resources and materials, including reagents, samples, instrumentation, computer resources, and analysis tools. All authors revised the article and read and approved the final version before submission. DF (corresponding author) and MRR (study statistician) had full access to and verify all the data in the study, and they had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

RNA-seq data from this study are available under Gene Expression Omnibus accession number GSE176005. All datasets generated during the current study are available from the corresponding author on reasonable request.

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