

Adipose-Derived Regenerative Cell Transplantation for the Treatment of Hand Dysfunction in Systemic Sclerosis: A Randomized Clinical Trial

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Objective. Hand dysfunction is common in systemic sclerosis (SSc). We undertook this study to evaluate the capacity of autologous adipose-derived regenerative cells (ADRCs) to improve hand function in SSc patients.

Methods. The Scleroderma Treatment with Celution Processed Adipose Derived Regenerative Cells Trial was a prospective, randomized, double-blind trial of ADRCs, in which ADRCs were obtained from patients with SSc by small-volume adipose tissue harvest, and the fingers of each patient were injected with ADRCs. The primary end point was change in hand function at 24 and 48 weeks, assessed using the Cochin Hand Function Scale (CHFS). One of the secondary end points included the change in Health Assessment Questionnaire disability index (HAQ DI) at 48 weeks. Separate prespecified analyses were performed for patients with diffuse cutaneous SSc (dcSSc) and those with limited cutaneous SSc (lcSSc).

Results. Eighty-eight patients were randomized to receive ADRCs ($n = 48$ [32 patients with dcSSc and 16 with lcSSc]) or placebo ($n = 40$ [19 patients with dcSSc and 21 with lcSSc]). Change in hand function according to CHFS score was numerically higher for the ADRC group compared to the placebo group but did not achieve statistical significance (mean \pm SD improvement in the CHFS score at 48 weeks 11.0 ± 12.5 versus 8.9 ± 10.5 ; $P = 0.299$). For patients with dcSSc, the between-group difference in the CHFS at 48 weeks was 6.3 points (nominal $P = 0.069$). For the secondary end point, the dcSSc group exhibited a between-group difference of 0.17 points in the HAQ DI (nominal $P = 0.044$) at 48 weeks. Of the ADRC-treated patients with dcSSc, 52% reported improvement greater than the minimum clinically important difference for both CHFS and HAQ DI compared to 16% in the placebo group (nominal $P = 0.016$). Small-volume adipose tissue harvest and ADRC treatment were well tolerated.

Conclusion. While the primary end point of this trial was not achieved, efficacy trends were observed in patients with dcSSc. Adipose tissue harvest and ADRC injection were demonstrated to be feasible. Further clinical trials of this intervention in the setting of dcSSc are warranted.

[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02396238) identifier: NCT02396238.

Some elements of the data reported herein were presented in poster form at the fifth Systemic Sclerosis World Congress held in Bordeaux, France, February 2018.

Supported by Cytori Therapeutics, Inc.

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Author disclosures are available at <https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42133&file=art42133-sup-0001-Disclosureform.pdf>.

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Submitted for publication November 10, 2021; accepted in revised form March 25, 2022.

INTRODUCTION

Impairment of hand function is universal in patients with systemic sclerosis (SSc) (1). While it is associated with less severe clinical symptoms than other complications of SSc (2), impaired hand function in SSc has a significant impact on quality of life, participation in the workforce, and activities of daily living (3,4). Despite the significance of hand dysfunction in SSc, there are few treatments with demonstrated effectiveness that specifically address this problem.

Human adipose tissue has been shown to be a rich source of cells with the potential to impact the inflammatory, vascular, and fibrotic sequelae of SSc (5,6). When isolated from adipose tissue using a standardized cell processing approach that meets standards for clinical use, this population of cells is referred to as adipose-derived regenerative cells (ADRCs) (7). Preclinical studies have shown that ADRCs promote increased vascularity and decreased fibrosis in a mouse model of SSc (5). An open-label, single-center trial of 12 patients reported data obtained following injection of ADRCs into the subcutaneous interdigital web space of the fingers of patients with moderate-to-severe hand dysfunction due to SSc (8–10). Substantial improvement in hand function was reported as early as 2 months after treatment and was then sustained for up to 3 years (10). In order to address the limitations of a single-arm, open-label trial, we conducted the Scleroderma Treatment with Celution Processed Adipose Derived Regenerative Cells (STAR) clinical trial using a randomized, double-blind, placebo-controlled design in which 88 SSc patients were enrolled.

PATIENTS AND METHODS

Study overview. The STAR trial was a prospective, randomized, double-blind, placebo-controlled, multicenter device trial to assess the safety and efficacy of ADRCs delivered by subcutaneous injection for the treatment of impaired hand function due to SSc. From May 2015 to September 2017, we enrolled male and female patients between ages ≥ 18 years and ≤ 70 years who had received a diagnosis of SSc according to the 2013 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for SSc (11) and whose diagnosis had been further subclassified as diffuse cutaneous SSc (dcSSc) with a duration > 5 years or limited cutaneous SSc (lcSSc) according to the Criteria for SSc Subsets (12). Patients also had to have moderate-to-severe hand dysfunction as evidenced by a Cochin Hand Function Scale (CHFS) score of ≥ 20 units (13,14). Full inclusion/exclusion criteria are available in the Supplementary Data (available on the *Arthritis & Rheumatology* website at <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>). Patients were permitted to continue use of systemic steroids and immunosuppressant medications provided that dosing was stable and within prespecified limits. This study was conducted in accordance with the Declaration

of Helsinki. Institutional Review Board approval was obtained at each study site and all patients signed informed consent.

Randomization. Patients were randomized via an interactive web response system into 1 of 2 parallel groups in a 1:1 ratio to receive either ADRCs or placebo. Patients were assigned the next available number in a computer-generated randomization schedule and received the treatment that corresponded to their randomization number. Randomization occurred after written informed consent had been obtained, after all screening procedures had been completed, after the subject's eligibility for the study had been confirmed, and just prior to the start of the liposuction procedure. A Consolidated Standards of Reporting Trials (CONSORT) flow chart showing subject enrollment and analysis is shown in Supplementary Figure 1 (available on the *Arthritis & Rheumatology* website at <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>).

Preparation and injection of ADRCs. After screening and randomization, patients underwent small-volume (100–360 ml) tumescent liposuction using manual aspiration under local anesthesia with or without conscious sedation. Tissue was then processed using the automated Celution System (Paracrine) according to the manufacturer's instructions. Briefly, tissue was injected into the processing chamber, washed, mixed with Celase GMP (a blended enzymatic reagent; Cytori Therapeutics), and continuously mixed at -37°C for 20 minutes. The ADRCs were then pumped to a centrifuge chamber where they were concentrated and washed. ADRCs were released for injection if they met cell viability and dose limits and had a negative gram stain. An aliquot of the cell and placebo product was sent for additional bacterial testing using the BACT/ALERT system (BioMérieux). Cell count and viability were determined using the NucleoCounter NC-100 automated cell counting system (Chemometec). In order to maintain blinding, a nonblinded technician who was not involved with patient enrollment, care, or outcome assessment prepared a placebo syringe containing lactated Ringer's solution visually matched to the ADRCs by the addition of 0.1–0.2 ml of the patient's blood. With the sole exception of this technician, all sponsor and study personnel and participating staff remained blinded to the treatment groups throughout the procedure and for the full duration of the study. Under procedural conditions of conscious sedation, neuroleptanalgesia, or topical analgesia, patients received injections of ADRCs (4 million cells per finger) or placebo in the subcutaneous space along the neurovascular bundle on each side of each finger (0.5 ml/injection) using a 25-gauge needle.

End points. The primary end point was change in CHFS at 24 and 48 weeks. The CHFS is a validated patient-reported outcome for assessment of hand function in SSc. The instrument assesses 5 domains rated from 0 (no problems performing the

task) to 5 (impossible to perform), with a total score ranging from 0 to 90. Two secondary end points were prespecified: change in the Health Assessment Questionnaire disability index (HAQ DI) at 48 weeks and change in the Raynaud's Condition Score (RCS) at 48 weeks. The HAQ DI is a validated instrument for the assessment of disability (15,16). The final score ranges from 0 to 3, with higher scores indicating greater disability. The RCS is a diary-based tool in which patients record a score that best indicates the extent of difficulty living with Raynaud's phenomenon each day (score range 0–10) (17). The RCS was the mean score recorded for the 14 days prior to the study visit.

Exploratory end points included change from baseline in CHFS, RCS, and HAQ DI scores across various time points. Other exploratory end points included change from baseline scores for the following assessments: the EuroQol 5-domain questionnaire (EQ-5D) for assessment of general health-related quality of life (18), patient and physician global assessments of SSc (captured using a 0–10-cm visual acuity scale for activity, damage, and overall patient health), hand corner distances (first corner is the distance between the tips of the thumb and index finger at maximum hand extension, second corner is the distance from the tips of the index and small fingers at maximum hand extension), grip and pinch strength assessed using a dynamometer, Modified Rodnan Skin Thickness Score (19) assessing only the hand at 3 sites (the back of each hand and the first and second phalanges of the most affected finger in each hand), hand volume (20), finger circumference, and finger ulcer counts. The statistical analysis plan prespecified separate analysis of data from all patients and subset analysis by disease subtype (dcSSc and lcSSc). Safety was assessed throughout the study by recording adverse events, which were mapped into MedDRA version 17.1 by System Organ Class and Preferred Terms.

Statistical analysis. In a pilot trial of autologous adipose tissue-derived stromal vascular fraction cell injections in patients with SSc (9), the mean \pm SD change in CHFS score from baseline to 6 months was mean \pm SD 27.3 \pm 17.2 units. A power calculation showed that a sample size of 41 patients per treatment group was needed to provide 90% power to detect a between-group difference of ≥ 13.7 points in the primary end point ($\alpha = 0.025$; to adjust for assessment at both 24 and 48 weeks), assuming that 50% of the 27.3-point response on the CHFS observed in the pilot was attributable to the placebo effect.

Safety and efficacy end points were summarized by treatment group using descriptive statistics for quantitative variables, and frequencies/percentages were used for categorical variables. Between-group comparisons were performed using analysis of covariance models with effects for the baseline value of the variable. Time to event (time to formation of a new finger ulcer) was determined using the Kaplan-Meier method with log rank testing and hazard ratios. Post hoc analyses of responder rates were performed using Fisher's exact test. Except where explicitly

stated, no adjustments for multiple comparisons were made to the *P* values of secondary or exploratory end points. All data described herein are from the intent-to-treat data set.

The minimum clinically important difference (MCID) for the CHFS instrument in patients with dcSSc who had significant hand dysfunction (baseline CHFS score ≥ 20) and dcSSc was determined from the STAR population baseline scores using the distribution method (21,22). The reliability of the CHFS in patients with SSc has previously been reported as 0.97 (14). A multiplier of 1.645 was applied to determine 95% confidence intervals (95% CIs) around the mean change in score. As a sensitivity analysis, the MCID was also calculated using the anchor method with change in the HAQ DI as an anchor (22,23). These 2 approaches derived MCID values of 9.5 and 9.9, respectively. Given the ordinal nature of the CHFS, patients were considered to have met the MCID threshold if the change from baseline was ≥ 10 points.

RESULTS

Baseline characteristics. A total of 105 patients were screened, and 88 patients were enrolled in the trial. Forty patients were randomized to receive placebo (19 patients with dcSSc and 21 with lcSSc), and 48 patients were randomized to receive ADRCs (32 patients with dcSSc and 16 with lcSSc) (Supplementary Figure 1, available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>). All patients completed the 24-week follow-up, with only 1 patient (ADRC group) not completing the final visit at week 48. The majority (85%) of the patients included in the study were women. The mean age of all patients randomized to receive a treatment was 53 years, and the mean disease duration was 13 years (Table 1). Patient demographic characteristics are shown in Table 1, and additional data are shown in Supplementary Table 1 (available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>). Except for the greater frequency of dcSSc in the ADRC-treated group (67% versus 48% for the placebo-treated group), the 2 treatment groups were well balanced.

Primary end point. The primary end point of this trial was not met (Figure 1 and Table 2). Specifically, whereas ADRC treatment was associated with numerically greater improvement in the CHFS score compared to that in the placebo group, the between-group difference was not statistically significant at either week 24 or week 48 among all patients or in either of the prespecified SSc subgroups. The greatest numeric difference in the CHFS score at week 48 between the placebo-treated group and the ADRC-treated group was in the subgroup of patients with dcSSc, in which a between-group difference in CHFS score of 6.3 points (95% CI $-0.5, 13.1$) was seen at 48 weeks (nominal *P* = 0.069) (Table 2 and Figure 1C). Post hoc analysis showed that 58% of patients (18 of 31) with dcSSc who were treated with ADRCs exhibited improvement in the CHFS score at week 48 that was

Table 1. Demographic and clinical characteristics of patients with SSc enrolled in the STAR trial*

	Placebo group	ADRCs group
No. of patients	40	48
Women	35 (88)	40 (85)
Age, mean \pm SD years	52 \pm 12	54 \pm 9
Disease duration from SSc onset, mean \pm SD years	13.3 \pm 8.9	12.7 \pm 7.9
Duration of Raynaud's phenomenon from SSc onset, mean \pm SD years	15.4 \pm 10.5	14.7 \pm 9.5
Patients with dcSSc	19 (48)	32 (67)
Patients with digital ulcers at baseline	13 (33)	17 (35)
Ethnicity or race		
Asian	1 (3)	0 (0)
Black or African American	4 (10)	1 (2)
White	34 (85)	47 (98)
Other	1 (3)	0 (0)
Prior treatment with systemic glucocorticoid prednisone	7 (18)	4 (8)
Antinuclear antibodies		
Positive	23 (58)	26 (54)
Negative	7 (18)	4 (8)
Not known	10 (25)	18 (38)
Antitopoisomerase antibodies		
Positive	9 (23)	9 (19)
Negative	19 (48)	20 (42)
Not known	12 (30)	19 (40)
Anticentromere antibodies		
Positive	6 (15)	2 (4)
Negative	19 (48)	22 (46)
Not known	15 (38)	24 (50)
Anti-RNA polymerase III antibodies		
Positive	4 (10)	3 (6)
Negative	8 (20)	12 (25)
Not known	28 (70)	33 (69)

* Except where indicated otherwise, values are the number (%) of patients. SSc = systemic sclerosis; STAR trial = Scleroderma Treatment with Celution Processed Adipose Derived Regenerative Cells trial; dcSSc = diffuse cutaneous SSc.

greater than the MCID, compared to 26% of patients (5 of 19) in the placebo group (nominal $P = 0.042$ for between-group difference).

Secondary end points. While the trial did not meet the primary end point, further analyses were performed in order to provide insights that might guide future studies of ADRCs for the treatment of

hand dysfunction in SSc. These assessments should be deemed exploratory and the corresponding P values (which have not been corrected for multiple comparisons) viewed as nominal.

The secondary end point (improvement from baseline in HAQ DI at week 48) was numerically greater for the ADRC-treated group than for the placebo-treated group among the overall population (Table 3). This between-group difference was most notable

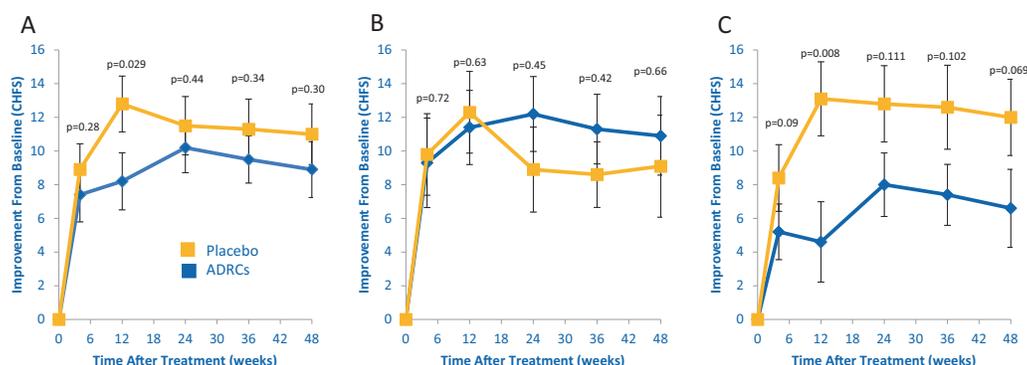


Figure 1. Change in the Cochin Hand Function Scale (CHFS) score over 48 weeks in systemic sclerosis (SSc) patients treated with adipose-derived regenerative cells (ADRCs) compared to SSc patients treated with placebo. Patients were evaluated overall (A) and after stratification into subgroups with limited (B) or diffuse (C) cutaneous SSc. Bars show the mean \pm SD change from baseline (in units).

Table 2. Change from baseline in CHFS score in patients receiving either ADRCs or placebo*

	All patients		Patients with lcSSc		Patients with dcSSc	
	Placebo	ADRCs	Placebo	ADRCs	Placebo	ADRCs
No. of patients	40	48†	21	16	19	32†
CHFS score						
Baseline	42.1 ± 11.4	39.3 ± 10.5	40.7 ± 11.4	34.6 ± 12.3	43.6 ± 11.5	40.7 ± 9.4
24 weeks	31.9 ± 14.9	27.8 ± 13.4	28.5 ± 14.3	27.5 ± 13.9	35.6 ± 15.0	27.9 ± 13.4
48 weeks	33.2 ± 15.8	28.1 ± 13.8	29.8 ± 16.4	27.3 ± 16.0	36.9 ± 14.6	28.6 ± 12.8
CHFS score improvement						
Baseline to 24 weeks						
Mean	10.2 ± 9.4	11.5 ± 12.0	12.2 ± 10.2	8.9 ± 10.1	8.0 ± 8.2	12.8 ± 12.8
Difference vs. placebo (95% CI) [P]		1.80 (-2.8, 6.5) [0.442]		2.61 (-9.6, 4.4) [0.453]		5.35 (-1.3, 12.0) [0.111]
Baseline to 48 weeks						
Mean	8.9 ± 10.5	11.0 ± 12.5	10.9 ± 10.7	9.1 ± 12.1	6.6 ± 10.1	12.0 ± 12.8
Difference vs. placebo (95% CI) [P]		2.62 (-2.4, 7.6) [0.299]		-1.72 (-9.6, 6.2) [0.660]		6.3 (-0.5, 13.1) [0.069]

* *P* values are uncorrected (not corrected for multiple comparisons) and were determined using an analysis of covariance model, with treatment as the main effect and the baseline Cochin Hand Function Scale (CHFS) score as the covariate. Except where indicated otherwise, values are the mean ± SD. 95% CI = 95% confidence interval; ADRCs = adipose-derived regenerative cells; lcSSc = limited cutaneous systemic sclerosis; dcSSc = diffuse cutaneous SSc.

† One patient with dcSSc in the ADRC-treated group did not complete the week 48 visit, thus reducing sample size for that visit to 47 overall and 31 for the dcSSc subgroup.

for the prespecified subgroup of patients with dcSSc (0.17 points [95% CI 0.04–0.38]) (nominal *P* = 0.044) (Table 3). This 0.17-point superiority of improvement in the HAQ DI in the ADRC-treated group compared to the placebo group was greater than has been previously reported as the MCID in the HAQ DI between groups in the setting of SSc (MCID of 0.14) (22). Post hoc analysis showed that 63% of the ADRC-treated patients with dcSSc exhibited improvements in HAQ DI scores at week 48 that were greater than the established MCID, compared to only 26% of patients in the placebo-treated group (nominal *P* = 0.019). Similarly, 47% of ADRC-treated patients with dcSSc showed improvements in HAQ DI scores at week 48 that were greater than the threshold indicating at least moderate improvement (>0.25 points), compared to only 16% of patients with dcSSc in the placebo-treated group (*P* = 0.035).

There was considerable concordance between change in CHFS and change in HAQ DI for patients with dcSSc who were treated with ADRCs (*r* = 0.72 by Pearson's correlation test, *P* < 0.0001). As shown in Figure 2A, 52% of ADRC-treated patients with dcSSc exhibited improvement greater than the MCID for both the CHFS and HAQ DI, compared to 16% of patients with dcSSc who received placebo (nominal *P* = 0.0163).

No relevant differences in the other secondary end point (improvement in RCS at week 48) were observed for any subgroup (Table 3). Prespecified exploratory analysis of RCS at other time points suggested greater improvement at week 12 in the ADRC-treated group compared to the placebo-treated group for all patients (mean ± SD change from baseline in RCS 1.3 ± 2.0 versus 0.4 ± 2.9; *P* = 0.009), for patients with dcSSc (mean ± SD change from baseline in RCS 1.7 ± 1.7 versus 0.9 ± 1.8; *P* = 0.09), and for patients with lcSSc (mean ± SD change from baseline in RCS 2.3 ± 1.5 versus 1.3 ± 2.4; *P* = 0.022).

Exploratory end points. Assessment of the time course of change in CHFS score for the dcSSc subset suggested an early separation between the ADRC- and placebo-treated groups, such that 4 weeks after treatment there was a between-group difference of 4.5 points (95% CI -0.8, 9.7) (*P* = 0.09) (Figure 1C). Twelve weeks after treatment, the mean between-group difference approached the MCID (9.3 points [95% CI 2.6, 16]; *P* = 0.008) (Figure 1C). Numerically superior improvement in the ADRC treatment group at week 48 was evident in each of the CHFS domains (kitchen, dressing, bathroom, office, and miscellaneous) (Supplementary Figure 2A, <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>) and in the HAQ DI domains presumably related to hand function (activities, dressing, eating, grip, hygiene, and reach) (Supplementary Figure 2).

At week 48, numerically superior improvements in baseline EQ-5D questionnaire scores were observed in the ADRC-treated group compared to the placebo-treated group for all prespecified subgroups (all subjects, lcSSc, and dcSSc groups) (Table 3). The between-group differences in EQ-5D subdomain scores were larger in the ADRC-treated group for subdomains that presumably related more to hand function (e.g., self-care and usual activities) than for the anxiety/depression subdomain (Supplementary Figure 2C).

The changes in patient and physician assessments of SSc activity favored the ADRC-treated group (Table 3), with differences being more evident in patient assessments for all patients (*P* = 0.027) and for the dcSSc subgroup (*P* = 0.046). Further, 23% (7 of 31) of ADRC-treated patients with dcSSc reported an improvement in SSc activity of more than 2 points at week 48, compared to 0% (0 of 19) of patients in the placebo-treated group (*P* = 0.035). There was a moderate correlation between improvement in CHFS

Table 3. Changes and between-group differences in secondary and exploratory end points in SSc patients treated with either ADRCs or placebo*

End point	Placebo		ADRCs		Between-group difference in score improvement, baseline to 48 weeks (95% CI) [P]
	Baseline	48 weeks	Baseline	48 weeks	
HAQ DI (range 0–3)					
All patients	1.33 ± 0.56	1.22 ± 0.59	1.26 ± 0.47	1.04 ± 0.52	0.11 (–0.15, 0.37) [0.105]
Patients with lcSSc	1.21 ± 0.55	1.04 ± 0.59	1.23 ± 0.41	0.98 ± 0.49	0.07 (–0.33, 0.47) [0.587]
Patients with dcSSc	1.45 ± 0.56	1.41 ± 0.54	1.28 ± 0.50	1.07 ± 0.54	0.17 (0.04, 0.38) [0.044]
RCS (range 0–10)					
All patients	4.3 ± 2.4	3.7 ± 3.0	3.4 ± 2.1	2.5 ± 2.6	0.305†
Patients with lcSSc	4.7 ± 2.3	3.7 ± 2.8	3.3 ± 2.1	2.2 ± 3.0	0.404†
Patients with dcSSc	3.9 ± 2.5	3.6 ± 3.3	3.4 ± 2.2	2.7 ± 2.4	0.430†
EQ-5D (range 0.19–1.00)					
All patients	0.70 ± 0.15	0.64 ± 0.19	0.73 ± 0.17	0.77 ± 0.14	0.104 (0.050, 0.154) [0.0002]
Patients with lcSSc	0.68 ± 0.17	0.64 ± 0.17	0.70 ± 0.17	0.77 ± 0.11	0.091 (0.005, 0.177) [0.033]
Patients with dcSSc	0.71 ± 0.13	0.64 ± 0.22	0.74 ± 0.17	0.79 ± 0.15	0.116 (0.077, 0.163) [0.005]
Assessment of SSc activity (range 0–10)					
Patient assessments					
All patients	5.6 ± 2.5	5.5 ± 3.0	4.6 ± 2.3	3.6 ± 2.7	0.76 (0.04, 1.48) [0.027]
Patients with lcSSc	5.8 ± 2.6	5.3 ± 2.7	4.3 ± 2.1	3.5 ± 2.9	0.27 (–1.06, 1.60) [0.220]
Patients with dcSSc	5.5 ± 2.5	5.6 ± 3.3	4.7 ± 2.4	3.6 ± 2.6	1.19 (0.06, 2.32) [0.046]
Physician assessments					
All patients	4.0 ± 2.2	3.7 ± 2.3	3.5 ± 1.6	2.8 ± 1.7	0.38 (–0.18, 0.94) [0.122]
Patients with lcSSc	4.5 ± 2.1	3.8 ± 1.7	3.5 ± 1.5	2.7 ± 2.1	0.21 (–1.18, 1.60) [0.262]
Patients with dcSSc	3.4 ± 2.2	3.5 ± 2.8	3.5 ± 1.7	2.9 ± 1.6	0.67 (–0.62, 1.96) [0.165]

* *P* values were uncorrected (not corrected for multiple comparisons) and were determined using an analysis of covariance model with treatment as the main effect and the baseline score as the covariate. Except where indicated otherwise, values are the mean ± SD. Uncorrected *P* values of <0.05 were considered significant. SSc = systemic sclerosis; ADRCs = adipose-derived regenerative cells; 95% CI = 95% confidence interval; HAQ DI = Health Assessment Questionnaire disability index; lcSSc = limited cutaneous SSc; dcSSc = diffuse cutaneous SSc; RCS = Raynaud's Condition Score; EQ-5D = EuroQol 5-domain instrument.

† The 95% confidence intervals and *P* values are not reported for RCS data as no subgroup was associated with an uncorrected *P* value of <0.05 for this end point.

and improvement in patient assessment of SSc activity in patients with dcSSc who were treated with ADRCs ($r = 0.44$, $P = 0.014$).

The ability of the patient to fully open their hands was assessed using the sum of the distances from the tip of the thumb to the tip of the small finger at full extension (sum of the corner distances).

Among patients with dcSSc, between-group differences favored the ADRC-treated group, such that 4 weeks after treatment the sum of all corner distances for both hands decreased by ~5 mm in the placebo-treated group, compared to a mean improvement of 14.7 mm in the ADRC-treated group (nominal $P = 0.027$).

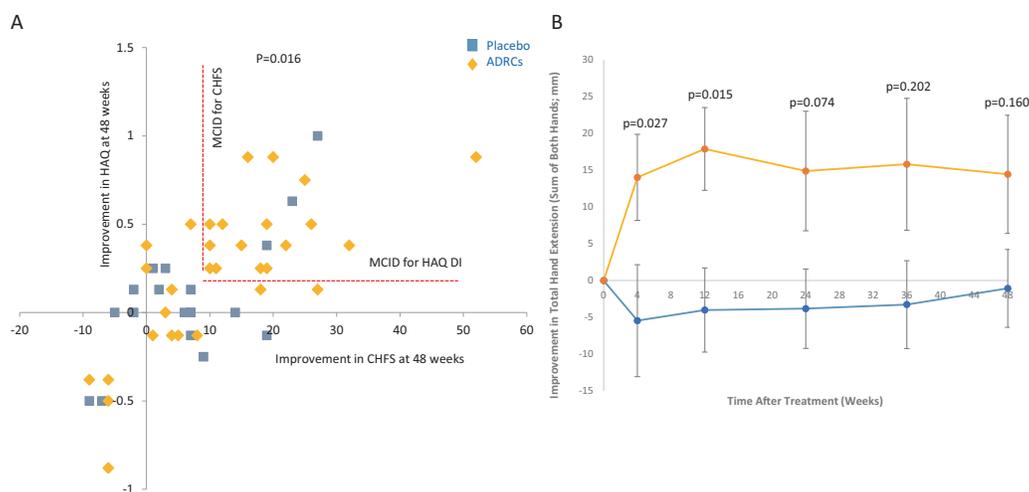


Figure 2. Change in hand function, as assessed by patient-reported outcome measures and hand extension, in patients with diffuse cutaneous SSc receiving ADRCs or placebo. **A**, Change from baseline in CHFS and Health Assessment Questionnaire disability index (HAQ DI) scores over 48 weeks. Symbols represent individual patients. **B**, Change in hand extension over 48 weeks. Positive numbers on y-axis indicate improvement and negative numbers indicate worsening. Bars show the mean ± SD. MCID = minimum clinically important difference. See Figure 1 for other definitions.

(Figure 2B). The mean absolute improvement of ~15 mm was sustained at week 12 (nominal $P = 0.015$) and at week 48 (14.1 mm), although the difference from the placebo-treated group at the later time point was no longer associated with a low nominal P value. No consistent, relevant differences were evident for grip or pinch strength for any subgroup (data not shown).

At baseline, the incidence of finger ulceration was higher in patients with dcSSc compared to patients with lcSSc (incidence 24% versus 16%; mean 3.25 ulcers/affected patient versus 2.0 ulcers/affected patient). ADRC treatment had no apparent effect on the healing of existing ulcers, but it was associated with reduction in the development of new ulcers in patients with lcSSc: 18.8% (3 of 16) of ADRC-treated patients with lcSSc developed new ulcers during the study compared to 52.4% (11 of 21) of placebo-treated patients with lcSSc.

Safety. Serious adverse events (SAEs) were reported in 1 patient (2.1%; 2 different SAEs) in the ADRC group and in 5 patients (12.5%; 8 different SAEs) in the placebo group. The patient in the ADRC group was hospitalized for respiratory symptoms with radiographic evidence of pneumonia, possibly due to aspiration, which was successfully treated with antibiotics. Given the use of conscious sedation, the event was deemed to be possibly related to either the liposuction or injection procedures. The same patient had another hospitalization for aspiration pneumonia 8 months later. SAEs in the placebo group included anemia secondary to a vaginal bleed, hypotension, joint effusion, angina, and upper gastrointestinal tract hemorrhage. All SAEs occurred more than 30 days after treatment and were not deemed to be related to study procedures. At least 1 AE was reported in 81.3% (39 of 48) of ADRC-treated patients and in 82.5% (33 of 40) of placebo-treated patients. Additional safety data are presented in Supplementary Tables 2–4, available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>.

AEs of any grade deemed potentially related to the liposuction procedure were reported in 3 patients (9%) in the ADRC group and 3 patients (16%) in the placebo group. The only SAE in the ADRC group was the aspiration event discussed above. All other events were deemed “mild” and included anemia, elevated transaminase levels, and abdominal wall hardness, and all resolved without sequelae within 21 days. AEs of any grade considered to be potentially related to the injection of ADRCs or placebo were reported in patients as follows: 6 (19%) in the ADRC group and 2 (11%) in the placebo group. One patient in the ADRC group reported moderate cellulitis in 1 finger, which resolved within 8 days without sequelae. All other events were deemed “mild” and included injection site swelling/discomfort, numbness, and edema. All mild events resolved within 36 days, with the exception of 1 ongoing case of mild numbness in the left index finger in 1 patient in the ADRC group. Finally, AEs regarded as potentially related to overall treatment were reported in patients as follows: 6 (19%) in the ADRC group and 1 (5%) in the placebo group. These included the injection-related events listed above

and a case of moderately decreased levels of hemoglobin in a patient in the ADRC group that resolved by day 8 of the study.

DISCUSSION

While SSc is notably a systemic disease (24), it has profound impacts on patient hand function, leading to substantial reductions in the ability to perform daily activities and in quality of life (1). Early data have been reported that suggest impaired hand function in SSc may be modified by direct application of autologous regenerative cells (5,9,10). We conducted a double-blind, randomized clinical trial in which autologous ADRCs or placebo were subcutaneously injected in the fingers of SSc patients with impaired hand function, in order to more rigorously assess the safety and efficacy of ADRCs. Although the primary end point of this trial was not met, the data suggest potentially important findings on the effects of treatment with ADRCs in patients with SSc. Specifically, it was found that improvement in hand function from baseline to 24 or 48 weeks in the ADRC-treated group was not statistically significantly different compared to that in the placebo-treated group among all patients or among the lcSSc or dcSSc subgroups.

The data are informative for both dcSSc and lcSSc subsets. Hand dysfunction in long-standing SSc (mean disease duration of 13 years in the current trial) is multifactorial, with contributions in varying degrees from skin thickening and resulting tethering, associated tendon shortening leading to claw hand deformity, involvement of upper extremity with large joint contractures, vascular complications such as Raynaud’s phenomenon and digital ulcers, calcinosis, and inflammatory arthritis. Use of the CHFS hand function score to assess treatment effects in patients with dcSSc was robust and reproducible (mean improvement of 12.8 units in the ADRC-treated group versus 8.0 units in the placebo-treated group) and may be related to a beneficial effect of ADRC treatment on severe skin thickening and tethering and the associated tendon shortening that was observed in the dcSSc subset. Our hypothesis was supported by the ability of patients to fully open their hands (a measure of hand dexterity), which was found to be more improved in dcSSc patients in the ADRC-treated group, as shown by a mean absolute improvement in the sum of the corner distances of ~15 mm that was seen at 12 weeks (nominal $P = 0.012$) and at 48 weeks (14.1 mm). Additionally, there was a greater change in hand dexterity in lcSSc patients who received placebo than in lcSSc patients who received ADRCs. This may have been related to milder skin thickening and tethering in this subset of patients (a known feature of lcSSc) and a higher placebo response. There were no differences in the baseline RCS and patient assessment of disease activity scores between the dcSSc and lcSSc subgroups that could explain the differences in hand function. ADRC treatment was associated with a reduction in the development of new finger ulcers in patients with dcSSc. In the subgroup of patients with dcSSc,

41% of patients in the ADRC group versus 53% in the placebo group developed finger ulcers, and in the subgroup of patients with lcSSc, 19% of patients in the ADRC group versus 52% in the placebo group developed finger ulcers without a beneficial impact on CHFS score.

Further analysis was performed to identify areas of interest that might guide the design of future studies. Post hoc assessments revealed that the percentage of ADRC-treated dcSSc patients exhibiting clinically meaningful improvement (improvement greater than the established MCID) at week 48 according to both the CHFS and HAQ DI instruments (each of which has been validated in SSc) was greater than the percentage of patients showing clinically meaningful improvement in the corresponding placebo-treated group (52% dual-response rate compared to 16% in the placebo group) (Figure 2). Furthermore, analysis of individual domains within the CHFS, HAQ DI, and EQ-5D instruments indicated that the greatest effects occurred in those parameters more obviously pertinent to hand function (e.g., self-care and usual activities) rather than those less directly related to hand function (e.g., walking and anxiety/depression) (Supplementary Figure 2, available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42133>). This improvement in subjective measures of hand function was associated with improvement in objective finger extension as evidenced by early and sustained improvements in the total distance between the fingers at maximum extension (sum of corner distances) (Figure 2B). Data for other exploratory end points showed trends that generally favored the ADRC-treated group, although the differences should be considered imprecise and exploratory.

The time course for each of these improvements in hand function following treatment (Figures 1 and 2B) was consistent with the findings demonstrated by Granel et al (9), with the maximum improvement evident by week 12. The durability of ADRC treatment was seen at up to 3 years in the study by Granel et al (9). In the current trial, the beneficial effect of ADRC treatment in the dcSSc subset was maintained over 48 weeks. There was no longer-term follow-up in the trial to assess continuing stability in CHFS scores. The data from the present study give us confidence in an effect that is attributable to ADRC treatment, and, if these results can be reproduced in another trial, we believe ADRCs will provide a durable treatment option for patients with dcSSc. One option for future studies is to repeat treatment at intervals of 12 or 24 weeks to see if this leads to additional sustained improvements or initial improvements for participants who did not experience a meaningful improvement in the symptoms of dcSSc earlier in the course of treatment.

A notable discrepancy between the current study and the pilot study was the absence of a sustained effect on RCS in the current study. The reason for this difference may lie in the substantially lower baseline RCS scores for patients in the current study compared to those in the pilot study (mean \pm SD baseline RCS score 4.2 ± 2.4 versus 7.2 ± 0.9). Furthermore, enrollment

in the current study was commenced in late spring and completed by fall; thus, most patients were not subjected to the extremes of winter during the study period, which may have impacted baseline symptoms and led to a ceiling effect in the RCS. Another notable difference from the prior open-label study was the absolute improvement from baseline in CHFS scores observed in the present study. ADRC-treated patients in the current trial exhibited improvement from baseline of ~ 12 points at 1 year. By contrast, the pilot study reported improvement of 24.2 points at 1 year. The reason for this difference is not clear but may reflect the absence of a placebo group in the pilot study, which might have inflated the patients' perception of anticipated benefits, thereby increasing an underlying placebo effect. Additionally, cultural differences between the study populations could have contributed to the treatment effect of the experimental agent: the open-label trial was conducted in France, and the double-blind trial was performed in the US. Finally, there may be differences in the severity/damage associated with hand dysfunction and the impact of the single-site versus multicenter nature of the current trial. For example, baseline CHFS score for all patients in the open-label study was ~ 50 points compared to a baseline CHFS score for all patients of ~ 40 points in the double-blind trial. As the current study was designed and statistical power was calculated using this earlier work, this likely led to significant underpowering of the present study.

The mechanisms by which ADRC treatment could yield clinical benefit in SSc are not fully understood. SSc is characterized by endothelial injury with a distinctive set of morphologic capillary microarchitecture changes (25) and changes consistent with chronic endothelial activation at the molecular level (26,27). Granel et al reported that treatment of SSc patients with ADRCs was associated with a reduction in avascular areas (vascular suppression score) at the nailfolds (9). Another study demonstrated that delivery of adipose stromal vascular fraction (a research form of ADRCs) led to improved vascularity in an animal model of SSc (5). These findings are consistent with several other preclinical studies showing improved blood vessel density and reduced inflammation with stromal vascular fraction/ADRCs (28–32). Additionally, these findings are also consistent with a report of improvement in hand function and reduced endothelial activation in a series of patients with SSc who received high-dose immunosuppressive therapy and autologous hematopoietic cell transplants (27). Given these reports, we hypothesize that ADRCs may act by elaboration of paracrine factors that lead to normalization of endothelial cell function with reduced capillary leakage, leukocyte infiltration, and improved angiogenesis. This hypothesis does not account for the absence of a treatment effect as assessed by the CHFS and HAQ DI for patients with lcSSc in the current study.

The data from the current clinical trial (and from the single-center, open-label pilot study) were obtained using a preparation of ADRCs that is very different from the population of cells

obtained by simply centrifuging adipose tissue, which is performed at different centers. Centrifugation of aspirated adipose tissue separates morsels of adipose cells from other cells collected during aspiration. As reported by Yoshimura et al (33), characterization of these cells by flow cytometry shows that the vast majority are simply cells from extravasated blood (CD45-positive white blood cells). This is expected, as liposuction does not break down the extracellular matrix that binds tissue and vascular cells together within the tissue morsels. By contrast, the process by which ADRCs are produced using the Celution System starts with the removal of blood cells prior to digestion with Celase. The cells concentrated by centrifugation of lipoaspirate are explicitly discarded during the production of ADRCs. Celase enzymatically digests the extracellular matrix, releasing vascular cells (endothelial, vascular smooth muscle cells, and pericytes), tissue macrophages, adipose tissue stromal (stem) cells, and blood cells trapped within vessels in the tissue morsels that may have a beneficial effect on SSc (34).

Consistent with the pilot study (9,10), the current trial showed an acceptable safety profile: adverse events were mild or moderate in both study groups. Adipose tissue collection was well-tolerated, with only the transient local pain and minor bruising expected from a small-volume aspiration, despite the generally lean nature of patients with SSc and their susceptibility to cutaneous ulceration. This was likely due to the small volume of adipose tissue required and the use of manual aspiration performed by experienced plastic surgeons without the use of general anesthesia or full sedation. Subcutaneous injection of ADRCs in the fingers was also well-tolerated, with only 1 SAE in the hand and finger osteomyelitis occurring in a patient in the placebo group ~5 months after injection. This safety profile is likely due to the nature of the system used to prepare the ADRCs in this study. The Celution System uses a sterile, functionally closed fluid pathway and a sterile, pharmaceutical-grade enzymatic reagent (Celase) that is washed out to levels that fall below defined safety thresholds during processing.

There is an unmet need for treatments that improve hand function limited by chronic skin and soft tissue sclerosis in patients with established dcSSc. The current study is unique in that the mean duration of skin induration was ~13 years. Any therapy that could improve activities of daily living in this subset of patients with established dcSSc would be a meaningful addition to rheumatologists' treatment regimens. The knowledge gained through this study can be used for other studies reporting positive results in patients with orofacial dysfunction due to SSc (35).

In conclusion, the current RCT demonstrated the feasibility and tolerability of small-volume adipose tissue harvest and cell injection into each finger in patients with SSc and hand dysfunction. While the prespecified primary end point (change from baseline in CHFS score) was numerically higher in the ADRC group, the differences did not achieve statistical significance in the full cohort of either the dcSSc or lcSSc subgroup. The between-group

differences were most prominent in the dcSSc group. While certain end points were associated with improvements that exceeded established MCIDs, we recognize that the results of this trial should be interpreted as encouraging and not definitive. Importantly, the data from the STAR trial should help facilitate study design and end point selection for an appropriately powered follow-up trial.

ACKNOWLEDGMENTS

We wish to thank the patients who participated in the STAR trial, the clinical research staff at each study site, Min Zhu of Cytori, and Dr. Guy Magalon of Marseille, France for his advice on the protocol. The STAR-1 trial investigators would like to thank the surgeons at each clinical site and the participants who contributed to this study.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Khanna had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Study conception and design. Khanna.

Acquisition of data. Khanna, Caldron, Martin, Kafaja, Spiera, Shahouri, Shah, Hsu, Ervin, Simms, Domsic, Steen, Hummers, Derk, Mayes, Chatterjee, Varga, Kesten, Fraser, Furst.

Analysis and interpretation of data. Khanna, Fraser.

ADDITIONAL DISCLOSURES

Author Fraser was employed by and held shares in Cytori Therapeutics during the time the study was conducted. He is currently an employee of JFK Consulting.

ROLE OF THE STUDY SPONSOR

Cytori Therapeutics facilitated the study design or collection, analysis, or interpretation of the data, but had no role in the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by Cytori Therapeutics.

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