ESC HEART FAILURE
ESC Heart Failure 2023; **10**: 1170–1183
Published online 13 January 2023 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.14281

ORIGINAL ARTICLE

Danish phase II trial using adipose tissue derived mesenchymal stromal cells for patients with ischaemic heart failure

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Abstract

Aims Patients suffering from chronic ischaemic heart failure with reduced left ventricular ejection fraction (HFrEF) have reduced quality-of-life, repetitive hospital admissions, and reduced life expectancy. Allogeneic cell therapy is currently investigated as a potential treatment option after initially encouraging results from clinical autologous and allogeneic trials in patients with HFrEF. We aimed to investigate the allogeneic Cardiology Stem Cell Centre Adipose tissue derived mesenchymal Stromal Cell product (CSCC_ASC) as an add-on therapy in patients with chronic HFrEF.
Methods and results This is a Danish multi-centre double-blinded placebo-controlled phase II study with direct intra-myocardial injections of allogeneic CSCC_ASC. A total of 81 HFrEF patients were included and randomized 2:1 to CSCC_ASC or placebo injections. The inclusion criteria were reduced left ventricular ejection fraction (LVEF $\leq$ 45%), New York Heart Association (NYHA) class II-III despite optimal anti-congestive heart failure medication and no further revascularization options. Injections of 0.3 mL CSCC_ASC (total cell dose 100×10^6 ASCs) (n = 54) or isotonic saline (n = 27) were performed into the viable myocardium in the border zone of infarcted tissue using the NOGA Myostar® catheter (Biological Delivery System, Cordis, Johnson & Johnson, USA). The primary endpoint, left ventricular end systolic volume (LVESV), was evaluated at 6-month follow-up. The safety was measured during a 3-years follow-up period.
Results Mean age was 67.0 ± 9.0 years and 66.6 ± 8.1 years in the ASC and placebo groups, respectively. LVESV was unchanged from baseline to 6-month follow-up in the ASC (125.7 ± 68.8 mL and 126.3 ± 72.5 mL, P = 0.827) and placebo (134.6 ± 45.8 mL and 135.3 ± 49.6 mL, P = 0.855) group without any differences between the groups (0.0 mL (95% CI −9.1 to 9.0 mL, P = 0.992). Neither were there significant changes in left ventricular end diastolic volume or LVEF within the two groups or between groups −5.7 mL (95% CI −16.7 to 5.3 mL, P = 0.306) and −1.7% (95% CI −4.4. to 1.0, P = 0.212), respectively). NYHA classification and 6-min walk test did not alter significantly in the two groups ($P > 0.05$). The quality-of-life, total symptom, and overall summary score improved significantly only in the ASC group but not between groups.
There were 24 serious adverse events (SAEs) in the ASC group and 11 SAEs in the placebo group without any significant differences between the two groups at 1-year follow-up. Kaplan–Meier plot using log-rank test of combined cardiac events showed an overall mean time to event of 30 ± 2 months in the ASC group and 29 ± 2 months in the placebo group without any differences between the groups during the 3 years follow-up period (P = 0.994).
Conclusions Intramyocardial CSCC_ASC injections in patients with chronic HFrEF were safe but did not improve myocardial function or structure, nor clinical symptoms.

Keywords Adipose tissue derived mesenchymal stromal cells; Allogeneic cell therapy; Heart failure; Ischaemic heart disease; Stem cell; Randomized clinical trial

Received: 10 September 2022; Revised: 10 December 2022; Accepted: 15 December 2022
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Introduction

Ischaemic heart disease (IHD) is one of the leading reason for hospital admissions and death.[1] IHD can result in heart failure with reduced quality of life, increased rate of hospital admissions and years of life lost. Autologous stem cell therapy in patients with IHD with and without heart failure has shown promising results but logistic obstacles make this form for interventional therapy difficult to be used in practice.[2–5] Thus, allogeneic stem cell therapy is investigated as a new treatment option in patients with IHD.

Different cell populations have been investigated for patients with IHD including mononuclear cells, bone marrow derived mesenchymal stromal cells (MSCs), $CD34^+$, $CD133^+$, endothelial progenitor cells from bone marrow or peripheral blood, and adipose tissue derived mesenchymal stromal cells (ASCs).[6–8] MSCs and ASCs are believed to regenerate ischaemic myocardium by paracrine activation of resident cells in the myocardium.[9–14] Previously, intramyocardial injections of autologous culture expanded MSCs in patients with IHD with and without ischaemic heart failure showed promising results.[15–20] Subsequently, intramyocardial injections of culture expanded autologous ASCs in patients with IHD with preserved left ventricular ejection fraction (LVEF $>$ 40%) did also show that the treatment was safe with a possible regenerative potential based on an increase in exercise capacity and reduced cardiac symptoms.[21,22]

To move forward clinically, a cryopreserved allogeneic ASC product (CSCC_ASC) was developed from healthy donors to be ready for delivery when needed.

To prepare for the present study, a pilot study was conducted using intramyocardial injections of allogeneic CSCC_ASCs in patients with ischaemic heart failure with reduced LVEF (HFrEF). The study demonstrated that this more homogenous standardized product was safe and logistically much easier to use clinically.[23]

The aim of the present study was to investigate the safety and efficacy of allogeneic CSCC_ASC in a multi-centre, single-country, phase II double-blinded, placebo-controlled study with intra-myocardial injections in patients with symptomatic chronic ischaemic HFrEF.

Methods

Study overview

This phase II study included patients from four hospitals in the greater area of Copenhagen, Denmark. All included patients were treated at Department of Cardiology, Rigshospitalet, Copenhagen in a 2:1 randomization with either intramyocardial injections of CSCC_ASC or placebo (saline) injections.

Screening and follow-up visits at 1, 3, 6, and 12 months after treatment were performed at the local cardiology departments.

The study complied with the Declaration of Helsinki and was approved by the Danish National Committee on Health Research Ethics (No. 1717872) and Danish Medicines Agency (1015050799). The study is registered at ClinicalTrials.gov (NCT03092284) and EudraCT (2015-001560-19). The local Good Clinical Practice Unit monitored the study throughout the entire study period.

The rationale, study design and inclusion/exclusion criteria have been reported previously.[23,24]

Study population

The study included 81 symptomatic chronic ischaemic HFrEF patients aged 30–85 years with reduced left ventricular ejection fraction (LVEF $\leq$ 45%), New York Heart Association (NYHA) class II-III, without further revascularization options. The patients were at the time of inclusion on maximal tolerable guideline-recommended anti-congestive heart failure medical treatment. Inclusion and exclusion criteria are shown in *Appendix A*.

CSCC_ASC production

CSCC_ASCs were produced in Cardiology Stem Cell Centre, Rigshospitalet University Hospital Copenhagen in an approved Good Manufacturing Practice (GMP) facility (manufacturing no. 23909 and tissue establishment no. 32298, issued by Danish authorities). The facility was inspected every second year by the Danish Medicines Agency and Danish Patient Safety Authority, and was in compliance with the EU Guidelines for GMP of Medicinal Products for Human Use (certificate of GMP compliance no. DK IMP 92217).

The ASCs were obtained from five healthy donors by abdominal liposuction in local anaesthesia for this study (Printzlau Private Hospital, Denmark). The donors were not previously known to have any diseases or were taking medication. Donors signed an informed consent in compliance with the Declaration of Helsinki. Thirty days prior to liposuction and on the day of donation, the donors were tested for HIV1/2, hepatitis B and C, syphilis, and HTLV I/II serology by serum analysis and by repeated serology and/or nucleic acid testing on the day of donation. The donors did not undergo any kind of genetic testing.

As previously described, the culture expansion of ASCs was performed in Quantum Cell Expansion Systems (Terumo, USA).[25–27] Prior to cell harvest, absence of mycoplasma was confirmed on all bioreactor expansions and absence of bacteria, fungi, and endotoxins was confirmed on the final cell product. Further release criteria were ASC viability (>80%) and identity by flow cytometry (stable positive markers CD90, CD105, and CD73; and negative markers CD45 and HLA-DR). Final product, CSCC_ASC constituted 110 million ASCs/5 mL CryoStor CS10 (BioLife Solutions).[23,28,29] The cells were not exposed to any type of preconditioning. They were ready for injection after 5–10 min thawing.

A patient randomized to the active arm, was treated with a vial of CSCC_ASC, which consisted of ASCs from only one donor. Patients randomized to placebo received injections of isotonic saline from a vial identical to the CSCC_ASC vials. The placebo and CSCC_ASC vials were stored in nitrogen dry-storage until use.

Cell injections

Prior to the injections of CSCC_ASC or placebo, a 3D map of left ventricle was created using the NOGA XP® system (Biological Delivery System, Cordis, Johnson & Johnson, USA).[18,22] Afterwards, the injections were performed using MYOSTAR® injection catheter (Biological Delivery System, Cordis, Johnson & Johnson, USA). The CSCC_ASCs or placebo was injected into the myocardium by 12–15 injections of 0.3 mL solution except the first injection, which consisted of 0.4 mL. Point by point measurement generates an electromechanical 3D LV map. The system distinguishes between viable (unipolar voltage >12 mV, bipolar voltage >2.5 mV, local linear shortening-LLS >6%), non-viable-myocardium (unipolar voltage <6 mV, bipolar voltage <1.5 mV, LLS <4%) and border zone (unipolar voltage 6–12 mV, bipolar voltage 1.5–2.5 mV, LLS 4%–6%) around myocardial scar tissue. To ensure appropriate injection into the ventricle wall, the injection catheter tip was located perpendicular to the ventricle wall and a ventricular extrasystole was elicited when extending the needle into the wall before any injection. The injections were performed into the viable myocardium judged by the 3D map in the border zone of infarcted tissue with a unipolar voltage >6 mV.

Endpoints

The *primary endpoint* was the difference between the two groups in left ventricular end-systolic volume (LVESV) from baseline to 6 months follow-up measured by echocardiography.[24]

Before enrolling patients into this trial, we made a power calculation based on our previous experiences.[16] Enrolment of 81 patients in a 2:1 randomization would detect a difference in LVESV of 13.0 mL with a 5% alpha value for a statistical power of 90% and with a standard error up to 15 mL.

The *secondary endpoints* were other echocardiography imaging related data, safety and clinical endpoints at 1, 3, 6 and 12 months after the treatment.[24]

Safety of CSCC_ASC treatment was registered as incidence and severity of serious adverse events (SAEs) and suspected unrelated serious adverse events at 12 months follow up.

Long-term safety outcome was assessed by a combined cardiac endpoint of death, hospitalization for worsening heart failure including inserting of a bi-ventricular pacemaker and hospitalization because of ventricular tachycardia or fibrillation 1, 2, and 3 years after treatment. Death and hospital admissions were retrieved from the Danish patient register in which all deaths and hospital admissions in Denmark are entered.

Echocardiography

Echocardiography was performed at baseline and 6 months after treatment. Images were obtained at parasternal and apical 2D views. All image data was stored on a central server and analysed blinded by two independent observers not aware of the treatment allocation. Moreover, they did not have access to each other's analysed images.

After the images were analysed by the two observers, the measurements were transferred to an independent third person. If the difference between the two observers was more than ±2 standard deviations in LVESV and left ventricular end-diastolic volume (LVEDV), images were re-analysed by the two observers. If the new analysis were within the original ±2 standard deviations, then the new measurements were accepted; if not, the third person analysed the images.

The mean values between the two/three observers of the measurements at baseline and follow-up were used for further statistical analysis.

Statistics

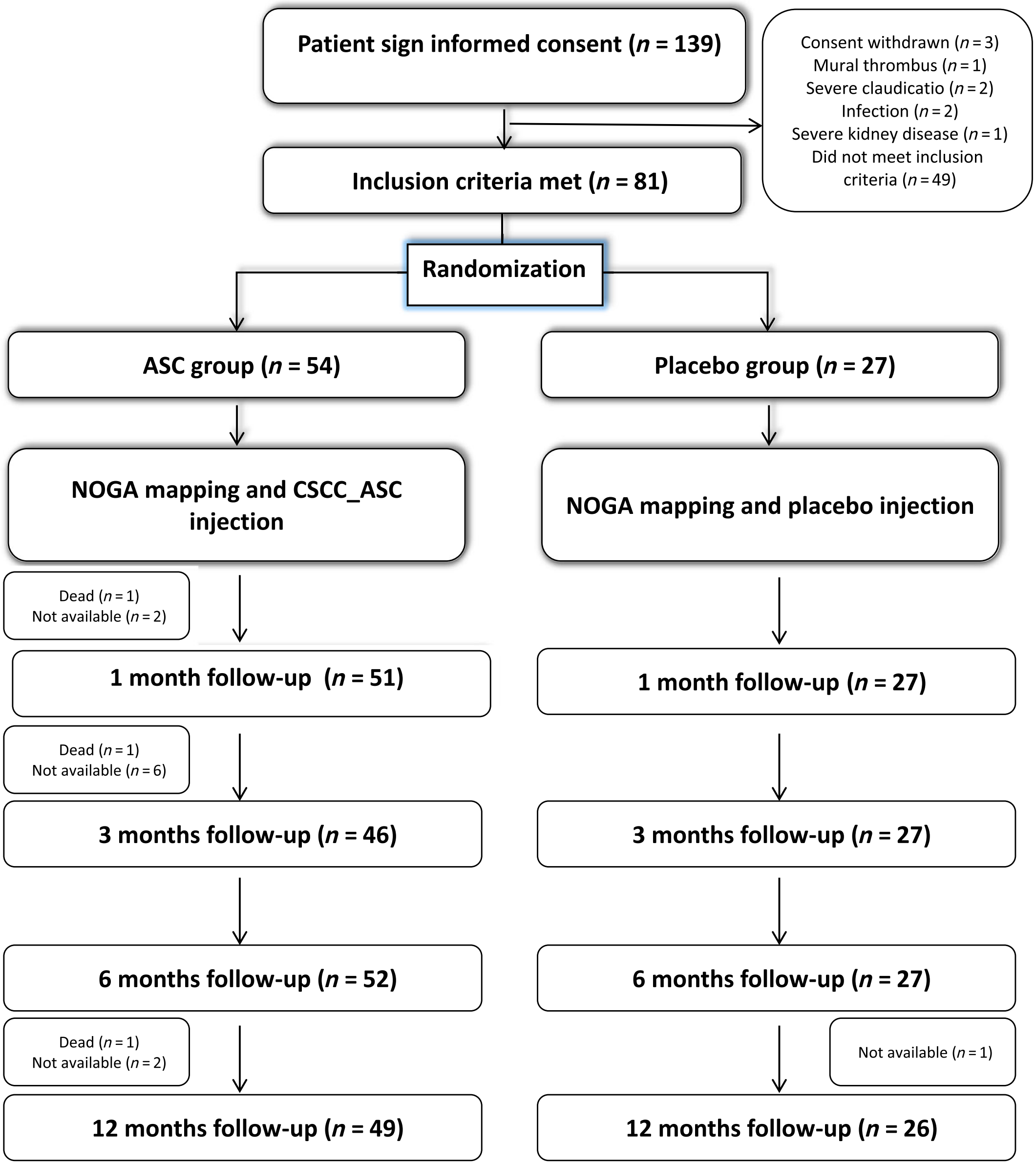
SPSS 25 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Paired t-test was used for normal distributed continuous data comparison within groups and unpaired t-test was used for comparison between groups. Pearson's χ^2 or Fisher's exact test were used for comparison of categorical data as appropriate. Repeated measure with autoregressive covariance structure was used for follow-up data with more than two time-points. Data were analysed as an intention-to-treat analysis. Kaplan–Meier curves using log-rank test was used to analyse the incidence of combined cardiac endpoint. Two-sided *P*-value of <0.05 was considered statistically significant.

Results

Overall, 67 men and 14 females were included and randomized 2:1 to either 100 × 10^6 ASCs (*n* = 54) or placebo injections (*n* = 27) (*Figure 1*).

The baseline characteristics are shown in *Table 1*. There were no major differences between the two groups except previous incidence of myocardial infarction, treatment with acetylsalicylic acid and diastolic blood pressure. The eight patients without a previous history of myocardial infarction had significant coronary atherosclerosis not amenable for revascularisation documented by a recent coronary angiography. The 6-min walking distance before treatment was 388 ± 92 m and 416 ± 121 m (mean ± SD) in the ASC and placebo group, respectively. At baseline, the LVESV was 125.7 ± 68.8 mL, the LVEDV was 184.7 ± 80.6 mL, and the LVEF was 34.2 ± 7.9% in the ASC group. In the placebo group, the LVESV was 134.6 ± 45.8 mL, the LVEDV was 193.6 ± 50.9 mL, and the LVEF was 31.4 ± 7.2% at baseline.

Figure 1 Overview of the study.



DOI: 10.1002/ehf2.14281

Table 1 Baseline characteristics

Parameter	ASC group (n = 54)	Placebo group (n = 27)	P-value
Patient profile			
Age (years)	67.0 ± 9.0	66.6 ± 8.1	0.834
Gender (male)	44 (81.5)	24 (88.9)	0.528
Smoking			
Current	9 (16.7)	4 (14.8)	1.000
Former	35 (64.8)	15 (55.6)	0.678
Diabetes mellitus			
Type I	2 (3.7)	1 (3.7)	0.776
Type II	12 (22.2)	7 (25.9)	0.736
Stroke	9 (16.7)	2 (7.4)	0.321
TCI	2 (3.7)	1 (3.7)	1.000
PAD	6 (11.1)	1 (3.7)	0.415
Pulmonary disease	8 (14.8)	3 (11.1)	0.744
BMI (kg/m^2)	28.8 ± 5.1	26.9 ± 4.3	0.088
Blood pressure			
Systolic (mmHg)	118 ± 18	111 ± 13	0.074
Diastolic (mmHg)	73 ± 11	68 ± 8	0.026
Heart rate (b.pm.)	66 ± 9	66 ± 12	0.970
Cardiac history			
Previous MI	46 (85.2)	27 (100.0)	0.047
Previous PCI	32 (59.3)	21 (77.8)	0.099
Previous CABG	31 (57.4)	11 (40.7)	0.157
Hypertension	35 (64.8)	15 (55.6)	0.678
Hypercholesterolemia	46 (85.2)	23 (85.2)	0.761
Family history of premature IHD	18 (33.3)	12 (44.4)	0.267
Cardiac device implant	41 (75.9)	23 (85.2)	0.335
Cardiac valve operation	2 (3.7)	1 (3.7)	1.000
Baseline endpoints			
NYHA class	2.2 ± 0.4	2.3 ± 0.4	0.576
CCS class	1.7 ± 0.8	1.3 ± 0.6	0.553
LVESV (mL)	125.7 ± 68.8	134.6 ± 45.8	0.544
LVEDV (mL)	184.7 ± 80.6	193.6 ± 50.9	0.603
LVEF (%)	34.2 ± 7.9	31.4 ± 7.2	0.131
6-min walking test (m)	388 ± 92	416 ± 121	0.253
Medication			
Acetylsalicylic acid	44 (81.5)	15 (55.6)	0.013
Clopidogrel	10 (18.5)	4 (14.8)	0.765
Prasugrel	1 (1.9)	1 (3.7)	1.000
Ticagrelor	5 (9.3)	3 (11.1)	1.000
Warfarin	8 (14.8)	5 (18.5)	0.751
Angiotensin-converting enzyme inhibitors	24 (44.4)	13 (48.1)	0.752
Angiotensin II receptor blockers	19 (35.2)	13 (48.1)	0.261
Beta-blocker	49 (90.7)	27 (1.0)	0.164
Calcium antagonist	4 (7.4)	0 (0.0)	0.296
Diuretic agent	44 (81.5)	20 (74.1)	0.440
Aldosterone receptor blockers	17 (31.5)	13 (48.1)	0.143
Statins	48 (88.9)	26 (96.3)	0.415
Non-statin lipid lowering drug	5 (9.3)	4 (14.8)	0.472
Nitrate	9 (16.7)	4 (14.8)	1.000
Insulin	5 (9.3)	4 (14.8)	0.472
Liraglutide	2 (3.7)	1 (3.7)	1.000
Oral anti-diabetic	11 (20.4)	2 (7.4)	0.201
Biochemical profile			
NT-ProBNP (pmol/L)	163.5 ± 181.9	151.8 ± 142.7	0.771
CK-MB (μg/L)	3.0 ± 1.5	2.9 ± 1.4	0.652
Troponin T (ng/L)	21.5 ± 24.0	16.8 ± 7.0	0.324
Total cholesterol (mmol/L)	3.8 ± 1.0	3.8 ± 0.8	0.892
LDL-C (mmol/L)	1.9 ± 0.8	1.9 ± 0.6	0.831
HDL-C (mmol/L)	1.2 ± 0.4	1.3 ± 0.5	0.565
Triglycerides (mmol/L)	1.5 ± 0.9	1.5 ± 0.7	0.950
Creatinine (μmol/L)	103.3 ± 32.1	98.2 ± 22.2	0.466
eGFR (mL/min/1.73 m^2)	65.7 ± 18.2	69.4 ± 16.0	0.375
CRP (mg/L)	4.5 ± 8.4	2.2 ± 3.2	0.076

Note: Values are mean ± SD or n (%).
Abbreviations: ASC, adipose tissue derived mesenchymal stromal cells; BMI, body mass index; CABG, coronary artery bypass graft; CCS, Canadian Cardiovascular Society; CK-MB, creatine kinase myocardial band; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; IHD, ischaemic heart disease; LDL-C, low-density lipoprotein cholesterol; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; MI, myocardial infarction; NT-ProBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; PAD, peripheral arterial disease; PCI, percutaneous coronary intervention; TCI, transient cerebral ischaemia.

DOI: 10.1002/ehf2.14281

Safety

There were 24 SAEs in the ASC group and 11 SAEs in the placebo group without any significant differences between the two groups at 1-year follow-up (*Table 2*).

Three patients died during the first follow-up year. One patient died 1 month after treatment due to ventricular fibrillation, while another patient died 4 months after treatment due to stroke and the third patient died 8 months after treatment due to pulmonary embolism.

Kaplan–Meier plot using log-rank test did not show any differences between the groups in the combined cardiac endpoint in a 3 years follow-up period ($P = 0.994$) (*Figure 2*). The overall mean time to event was 30 ± 2 months in the ASC group and 29 ± 2 months in the placebo group.

Echocardiography

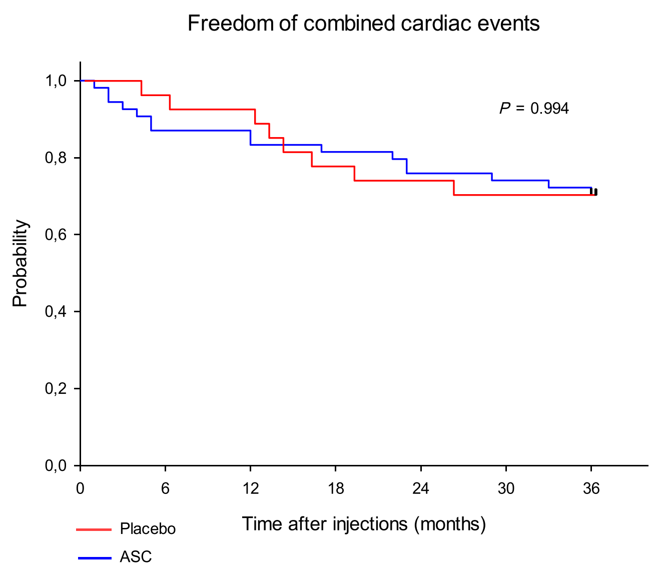
The primary endpoint LVESV was unchanged from baseline to 6 months follow-up within both the ASC (125.7 ± 68.8 mL and 126.3 ± 72.5 mL, $P = 0.827$) and placebo (134.6 ± 45.8 mL and 135.3 ± 49.6 mL, $P = 0.855$) groups. The difference between the two groups was 0.0 mL (95% CI −9.1 to 9.0 mL,

Table 2 Serious adverse events within 1-year follow-up

Serious adverse event	ASC group (n = 54)	Placebo group (n = 27)	*P*-value
Death	3 (5.6)	0 (0.0)	0.547
Hospitalizations:			
Heart failure worsening	5 (9.3)	2 (7.4)	1.000
Ventricular fibrillation/ tachycardia	1 (1.9)	1 (3.7)	1.000
Myocardial infarction	2 (3.7)	1 (3.7)	1.000
Atrial fibrillation	2 (3.7)	1 (3.7)	1.000
Angina worsening	5 (9.3)	1 (3.7)	0.658
Pneumonia	2 (3.7)	1 (3.7)	1.000
Urinary infection	3 (5.6)	0 (0.0)	0.547
Acute in chronic pancreatitis	0 (0.0)	1 (3.7)	0.333
ICD implantation	0 (0.0)	1 (3.7)	0.333
Occlusion femoral by-pass	0 (0.0)	1 (3.7)	0.333
Claudication	0 (0.0)	1 (3.7)	0.333
Cancer	1 (1.9)	0 (0.0)	1.000

Note: Values are n (%).
Abbreviations: ASC, adipose tissue derived mesenchymal stromal cells; ICD, implantable cardioverter-defibrillator.

Figure 2 Freedom of combined cardiac events. The combined cardiac endpoint consisted of death, hospitalization for worsening heart failure including inserting biventricular pacemaker and hospitalization because of ventricular tachycardia or fibrillation 1, 2, and 3 years after treatment.



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P = 0.992) (*Figure 3A*). Neither were there any significant changes in LVEDV or in LVEF within the two groups or between groups −5.7 mL (95% CI −16.7 to 5.3 mL, P = 0.306) and −1.7% (95% CI −4.4. to 1.0, P = 0.212), respectively (*Figure 3B,C*).

NYHA functional class, 6-min walk test, and Kansas City cardiomyopathy questionnaire

The NYHA class did not change from baseline to follow-up time points in the ASC or placebo group (*Figure 4A*). No differences between the two groups were observed.

In both the ASC and placebo groups, 6-min walking distance was unchanged from baseline to 3-, 6- and 12-month follow-up (*Figure 4B*). There were no significant differences between the groups at any time points.

The Kansas City Cardiomyopathy Questionnaire (KCCQ) overall summary score, total symptom score, quality of life, symptom frequency, and social limitation score increased significantly from baseline to follow-up time points in the ASC group but not in the placebo group (*Appendix B*). There were no significant differences between the groups at follow-ups.

Biochemical response

Overall plasma N-terminal pro B-type natriuretic peptide (NT-ProBNP) remained unchanged in the ASC and placebo group during the one-year follow-up period (*Figure 4C*). However, there was a significant decrease in NT-ProBNP from baseline to 6 months follow-up in the ASC group (P = 0.038) and from baseline to 3 months follow-up in the placebo group (P = 0.014).

A significant decrease from baseline to 1 and 3 months follow-up in creatine kinase MB (CK-MB) was observed in the ASC group (P = 0.004 and P = 0.019, respectively) and CK-MB decreased significantly from baseline to only 3 months follow-up in the placebo group (P = 0.008). Troponin T did not change significantly during follow-up in the groups (*Figure S1*).

There were no significant changes in C-reactive protein in the ASC group, but a significant increase was observed in the placebo group during the follow-up (*Figure S1*). The kidney function measured by creatinine did not change significantly during the follow-up time in the ASC or placebo group (*Figure S1*).

Discussion

This is the largest national double-blinded, randomized, multi-centre, placebo-controlled trial published to date aimed to investigate the safety and efficacy of the allogeneic adipose-derived mesenchymal stromal cell product CSCC_ASC as an add-on therapy in patients with symptomatic chronic ischaemic HFrEF.

Autologous and allogeneic ASC therapy has previously shown to be safe with encouraging results on efficacy in patients with IHD.[2,21–23] Treatment with this cryopreserved cell product CSCC_ASC obtained from healthy donors and culture expanded xeno-free in automated closed bioreactor system demonstrated promising short- and long-term follow-up safety data.[23] Using allogeneic cells eliminates the influence of patient related factors on the number of cells reached after culture expansion when using autologous cell therapy.[30] However, we could not detect any beneficial effect on cardiac structure or function in these patients with long-term symptomatic chronic ischaemic heart failure. Moreover, the finding was supported by the lack of clinical beneficial effect on the patients exercise capacity and symptoms. This result was in opposition to results from our own and other groups in previous minor phase I and II clinical trials in patients with HFrEF.[2,31]

A significant decrease in NT-ProBNP from baseline to 6 months follow-up in the ASC group was observed (P = 0.038) but overall, there were no significant differences in NT-ProBNP during the one-year follow-up period in the ASC or placebo group. Additionally, a significant improvement in KCCQ scores was seen in the ASC group but not in the placebo group. This is a small study including patients with a chronic disease. The question is whether the selected primary outcome measure is relevant in this group of patients or another more relevant outcome can be chosen.[32]

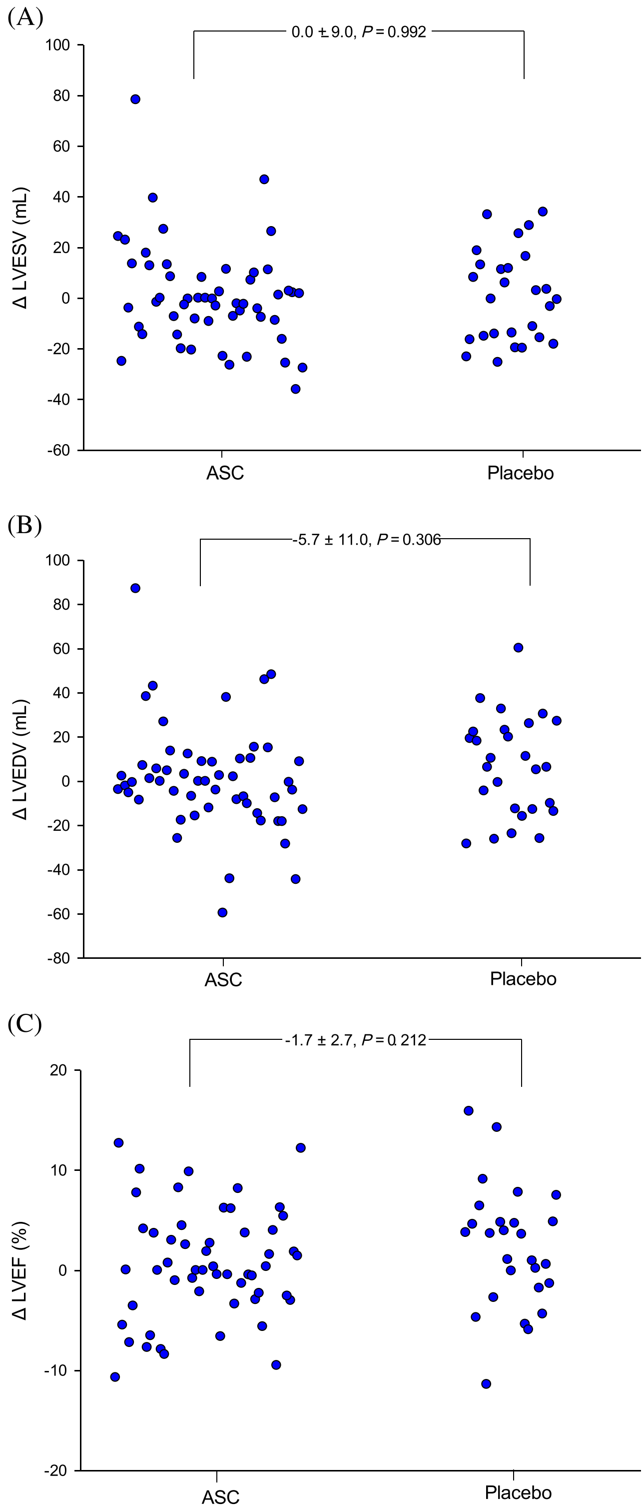
Based on the initial experience with the CSCC_ASC product in the safety trial, we planned the current trial, which turned out to be neutral clinically in its primary endpoint. The cell product is for every cell production tested for viability and functionality. Although viable, the cell product seems not to interact in a sufficient manner with the cardiac tissue to stimulate increase in cardiac function. Whether a higher number of cells or treatment more than once would be more effective is unknown.[33,34]

Although, an unequal randomization requires more patients to achieve same statistical power as in trials with equal randomization, we chose 2:1 randomization as we expected this group of patients preferring stem cell treatment and this would increase the adherence to the trial.

The mechanisms of actions for ASC regenerative capacity is through anti-apoptotic, antifibrotic, angiogenetic, and immunomodulatory processes, with evidence on immune activation being predominant in cardiac cell therapy.[35–37] Therefore, it can be speculated whether patients with an on-going inflammatory disease may benefit

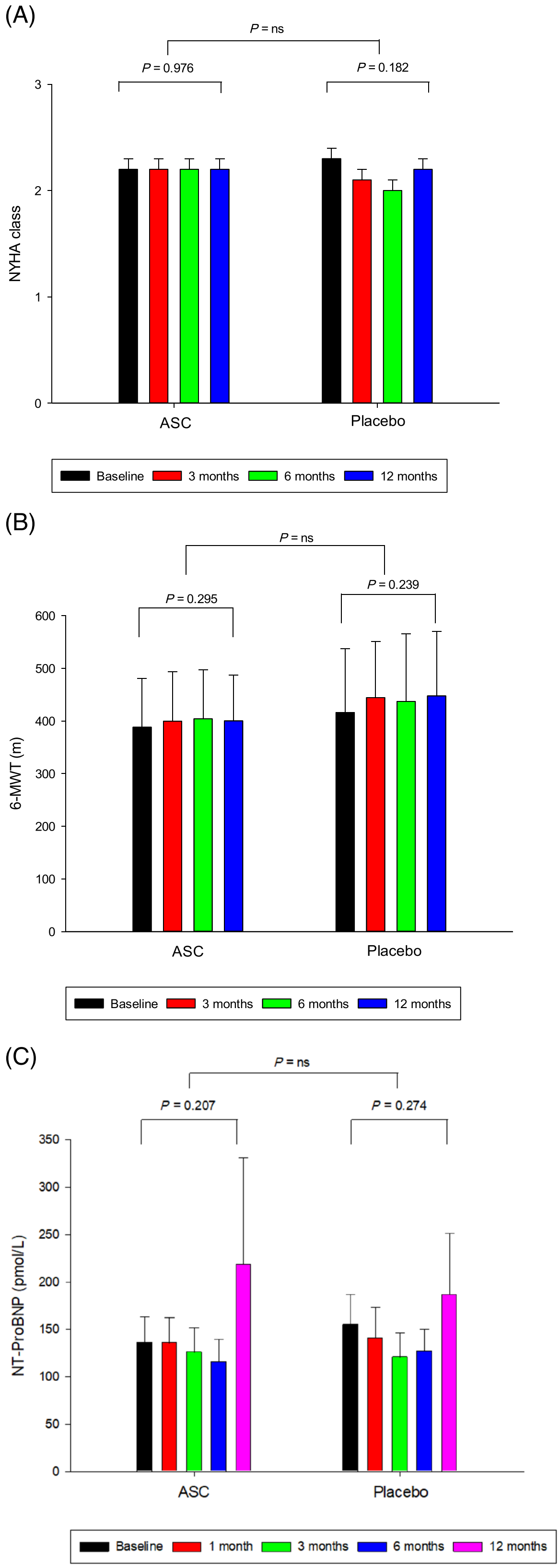
DOI: 10.1002/ehf2.14281

Figure 3 Difference between the two groups from baseline to 6-month follow-up in (A) left ventricular end-systolic volume (LVESV), (B) left ventricular end-diastolic volume (LVEDV), and (C) left ventricular ejection fraction (LVEF).



DOI: 10.1002/ehf2.14281

Figure 4 (A) NYHA classification, (B) 6-min walk test, and (C) NT-ProBNP at baseline, 3, 6- and 12-month follow-up.



from this therapy while the patients with a disease being in a chronic non-inflammatory state may not, due to the micro milieu in the myocardium.

These inflammatory processes are normally present in ischaemic myocardium.[36] In this study, the patients with chronic HFrEF most likely were a heterogeneous group having no to mild inflammatory activity in the myocardium. Thus, treatment of chronic HFrEF patients with no further treatment options may be too late to restore the cardiac function.[38] If that is the case, intervention at an earlier stage of the heart failure progression may improve the clinical outcome. Other factors have also been suggested to identify responders such as enlarged LVEDV at baseline.[39,40] These patients may experience a treatment effect.

Our findings are in accordance with the results presented from the placebo controlled DREAM-HF phase III trial with allogeneic bone marrow derived mesenchymal stem cells (rexlemestrocel-L) in patients with HFrEF.[41] The study did not reach its primary endpoint, the time to recurrent nonfatal heart failure-related major adverse cardiac events. However, it indicated that early treatment could be effectful since a sub-group of patients with NYHA class II had a 60% reduction in cardiac death and prevention of progression to NYHA class III.

The All-Star trial treated patients 4–12 months after a myocardial infarction with another allogeneic cell product, cardiosphere derived cells or placebo.[42] The study did not reach significance in the primary endpoint, reduction in scar tissue. However, it demonstrated an improvement in cardiac magnetic resonance measured cardiac ventricular functional parameters. This could be supportive for an early intervention in ventricular remodelling.

The cell product was delivered by direct injections into the myocardium using the NOGA XP® method. It is an ongoing discussion whether the cell retention within the heart is long enough to restore cardiac function.[43–45] Having the cell paracrine theory in mind, there must also be resident cells in the myocardium able to react with delivered cells.[13,46] However, if there are no or reduced number of resident cells and short cell retention time, then the injected cells will not be able to show their efficacy. As in other studies using allogeneic mesenchymal stromal cell, there were no Humane Leucocyte Antigen tissue type matching between the donor and the patients, which potentially could also play a role in the findings of this study. However, we and other groups have demonstrated that the delivered cells escape the immune system and normally not induce an anti-body reaction toward the donor cells and if so only in a very low transient concentration.[23]

In conclusion, this allogeneic adipose tissue derived mesenchymal stromal cell product CSCC_ASC stored as an off-the-shelf product is safe to inject directly into the myocardium. However, using CSCC_ASC as an add-on therapy in patients with symptomatic chronic HFrEF did not improve the primary endpoint LVESV or other secondary endpoints compared to placebo. Whether treatment at an earlier stage of heart failure or treatment more than once would be effective need to be investigated.

Acknowledgements

We would like to thank the study nurses Anne Lavigne and Louise Frandsen for their great enthusiasm and hard work to conduct and finalize the study. Mojdeh Lotfi, Kirstine Andreesen, and Rebekka Sørensen for their hard work in manufacturing of the stem cell product.

Conflict of interest

Jens Kastrup, Annette Ekblond and Mandana Haack-Sørensen are inventors of a granted patent (“STEM CELL THERAPY BASED ON ADIPOSE-DERIVED STEM CELLS” (WO2017068140A1 EP3365432A1) owned by the Capital Region of Denmark and Rigshospitalet, Copenhagen University Hospital, Denmark. The patent is granted in Europe and Australia. Applications are submitted in Canada, China, Hong Kong, Japan, Korea, and USA.

Jens Kastrup, Annette Ekblond, and Mandana Haack-Sørensen are founders of Cell2Cure ApS, which has a licence to commercialize the patent.

The authors declare no conflict of interest except the above mentioned.

Funding

This trial was supported by the Innovation Fund Denmark grant (CSCC grant no. 6153-00002A), Arvid Nilssons Foundation, Skibsreder Per Henriksen, R & Hustrus Foundation, and Aase and Ejnar Danielsens Foundation.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Change in (A) CK-MB, (B) Troponin T, (C) C-reactive protein and (D) creatinine during follow-up time.

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Appendix A: Inclusion and exclusion criteria

Inclusion criteria:

1. 30–85 years of age
2. Signed informed consent
3. Chronic stable ischaemic heart disease
4. Symptomatic heart failure (NYHA II-III)
5. Left ventricular ejection fraction ≤45% documented by echocardiography, computed tomography or magnetic resonance imaging obtained after up-titration in heart failure medication (if cardiac resynchronisation therapy device (CRT) 3 months after operation)
6. Plasma NT-pro-BNP > 300 pg/mL (>35 pmol/L) in sinus rhythm and plasma NT-pro-BNP > 422 pg/mL (> 49 pmol/L) in patients with atrial fibrillation
7. Maximal tolerable heart failure medication
8. Heart failure medication unchanged 2 months prior to inclusion/signature of informed consent. Changes in diuretics accepted.
9. No option for percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG)
10. Patients who have had PCI or CABG within 6 months of inclusion must have performed a new angiography less than 1 month prior to inclusion and at least 4 months after the intervention to rule out early restenosis

11. Patients cannot be included until 3 months after implantation of a CRT or 1 month after an implantable cardioverter device

Exclusion criteria

1. Heart failure class NYHA I or IV
2. Acute coronary syndrome with elevation of CKMB or troponins, stroke or transitory cerebral ischaemia within 6 weeks of inclusion
3. Other revascularisation treatment within 4 months of treatment
4. If clinically indicated the patient should have a coronary angiography before inclusion
5. Moderate to severe aortic stenosis (valve area <1.3 cm^2) or valvular disease with option for surgery
6. Diminished functional capacity for other reasons such as: obstructive pulmonary disease with forced expiratory volume in 1 second (FEV1) < 1 L/min, moderate to severe claudication, severe arthrosis or severe pain from the musculoskeletal system or morbid obesity
7. Clinically significant anaemia (haemoglobin < 6 mmol/L), leukopenia (leucocytes <2 × 10^9/L), leucocytosis (leucocytes >14 × 10^9/L) or thrombocytopenia (thrombocytes <50 × 10^9/L)
8. Anticoagulation treatment that cannot be paused during cell injections
9. Patients with reduced immune response
10. History with malignant disease within 5 years of inclusion or suspected malignity – except treated skin cancer other than melanoma
11. Pregnancy or lactation
12. Other experimental treatment within 4 weeks of baseline tests
13. Participation in another intervention trial
14. Known hypersensitivity to DMSO

Appendix B

Table B1. Kansas City Cardiomyopathy Questionnaire baseline and follow-up time points

		Baseline	3 months	6 months	12 months	*P*-value overall
Overall summary score						
	ASC	64 ± 3	70 ± 3	72 ± 3	72 ± 3	0.011
	Placebo	71 ± 3	72 ± 4	71 ± 4	71 ± 4	0.955
P-value from baseline to follow-up	ASC		0.012	0.002	0.003	
	Placebo		0.692	0.987	0.931	
Total symptom score						
	ASC	69 ± 3	74 ± 3	75 ± 3	77 ± 3	0.035
	Placebo	77 ± 3	77 ± 4	78 ± 4	78 ± 5	0.996
P-value from baseline to follow-up	ASC		0.026	0.032	0.004	
	Placebo		0.991	0.855	0.884	
Clinical summary score						
	ASC	68 ± 3	73 ± 2	74 ± 3	74 ± 3	0.067
	Placebo	75 ± 3	75 ± 4	77 ± 4	75 ± 4	0.818
P-value from baseline to follow-up	ASC		0.026	0.012	0.026	
	Placebo		0.779	0.499	0.910	
Quality of life						
	ASC	63 ± 3	69 ± 3	70 ± 3	71 ± 3	0.037
	Placebo	70 ± 5	72 ± 4	69 ± 5	67 ± 5	0.593
P-value from baseline to follow-up	ASC		0.014	0.015	0.014	
	Placebo		0.403	0.784	0.526	
Symptom frequency						
	ASC	67 ± 3	72 ± 3	73 ± 3	73 ± 3	0.045
	Placebo	74 ± 3	74 ± 4	75 ± 4	74 ± 4	0.992
P-value from baseline to follow-up	ASC		0.018	0.028	0.007	
	Placebo		0.864	0.934	0.966	
Social limitation						
	ASC	59 ± 4	64 ± 4	69 ± 4	69 ± 4	0.008
	Placebo	66 ± 5	69 ± 5	65 ± 5	69 ± 5	0.664
P-value from baseline to follow-up	ASC		0.135	0.007	0.003	
	Placebo		0.283	0.883	0.434	

(*Continues*)

		Baseline	3 months	6 months	12 months	*P*-value overall
Physical limitation						
	ASC	66 ± 3	72 ± 3	72 ± 3	71 ± 3	0.239
	Placebo	73 ± 3	72 ± 5	76 ± 4	72 ± 4	0.527
P-value from baseline to follow-up	ASC		0.042	0.063	0.150	
	Placebo		0.583	0.243	0.687	
Symptom stability						
	ASC	52 ± 3	52 ± 3	53 ± 2	52 ± 3	0.940
	Placebo	46 ± 2	51 ± 2	57 ± 3	54 ± 3	0.047
P-value from baseline to follow-up	ASC		1.000	0.628	1.000	
	Placebo		0.022	0.019	0.018	
Symptom burden						
	ASC	72 ± 4	76 ± 3	78 ± 3	80 ± 4	0.085
	Placebo	80 ± 4	81 ± 4	81 ± 5	82 ± 5	0.991
P-value from baseline to follow-up	ASC		0.078	0.074	0.011	
	Placebo		0.878	0.802	0.764	
Self-efficacy						
	ASC	82 ± 3	87 ± 2	83 ± 3	85 ± 3	0.240
	Placebo	80 ± 3	83 ± 3	80 ± 3	80 ± 4	0.579
P-value from baseline to follow-up	ASC		0.040	0.664	0.305	
	Placebo		0.207	0.882	0.901	

Note: Values are mean ± SD.
Abbreviation: ASC, adipose tissue derived mesenchymal stromal cells.