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HUMAN CLINICAL ARTICLE

Cryopreserved Off-the-Shelf Allogeneic Adipose-Derived Stromal Cells for Therapy in Patients with Ischemic Heart Disease and Heart Failure—A Safety Study

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Key Words. Clinical trial • Cardiac • Adipose stem cells • Cellular therapy • Mesenchymal stem cells • Tissue regeneration • Somatic cell therapy • Stromal cells

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ABSTRACT

The present first-in-human clinical trial evaluated the safety and feasibility of a newly developed and cryopreserved Cardiology Stem Cell Centre adipose-derived stromal cell (CSCC_ASC) product from healthy donors for intramyocardial injection in ten patients with ischemic heart disease and ischemic heart failure (IHF). Batches of CSCC_ASC were isolated from three healthy donors by liposuction from abdominal adipose tissue. Adipose mesenchymal stromal cells were culture expanded in bioreactors without the use of animal constituents, cryopreserved, and stored in vials in nitrogen dry-storage containers until use. Direct injection of CSCC_ASC into the myocardium did not cause any complications or serious adverse events related to either treatment or cell administration in a 6-month follow-up period. Four out of ten heart failure patients developed donor-specific de novo human leukocyte antigen (HLA) class I antibodies, and two out of ten patients had donor-specific HLA antibodies already at baseline. There were no clinical symptoms or changes in inflammatory parameters in the follow-up period that indicated an ongoing immune response. There was a tendency toward improvement in cardiac function after CSCC_ASC treatment at 6-month follow-up: left ventricular end systolic volume decreased and left ventricular ejection fraction increased. In addition, exercise capacity increased. These changes were independent of the presence or absence of HLA antibodies. It is concluded that the newly developed cryopreserved product CSCC_ASC from healthy donors was a safe and feasible treatment. We observed a tendency toward efficacy in patients with IHF. These findings have to be confirmed in larger placebo controlled clinical trials. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:1963–1971*

SIGNIFICANCE STATEMENT

This first-in-human study of an off-the-shelf cryopreserved Cardiology Stem Cell Centre adipose-derived stromal cell product from healthy donors demonstrated safety, feasibility, and a tendency toward clinical efficacy in ten patients with ischemic heart disease and heart failure. The presence of a ready-to-use cryo-stored cell product will eliminate many of the logistic barriers in disseminating cell therapy to many patient groups and will also reduce the cost of the treatments.

INTRODUCTION

Coronary artery atherosclerosis causing ischemic heart disease (IHD) is the most common cause of death, with more than 17 million deaths worldwide each year, and is a major cause of hospital admissions in industrialized countries [1]. Established therapies have reduced mortality of IHD significantly but have left an increasing number of symptomatic patients with chronic IHD and/or ischemic heart failure (IHF) without further treatment options.

Stem cell therapy with mesenchymal stromal cells, originating from different tissues, has emerged as a new regenerative therapeutic tool in this patient group as well as in several other debilitating diseases for which no cure is currently available. Clinical studies have been conducted

with autologous bone marrow-derived mesenchymal stromal cells (BMSCs) and adipose-derived stromal cells (ASCs) in patients with IHD and IHF [2–4]. The treatments have been safe and the efficacy has been promising.

Our group has completed three clinical trials with fresh culture expanded autologous BMSCs and ASCs of approximately 150 patients with chronic IHF and IHD [5–9]. From these studies, we have realized, that the use of autologous cells is highly complex and hampers standardization and smooth logistics in this treatment modality: autologous BMSCs and ASCs must be culture expanded from each individual patient in order to reach an adequate amount of cells for treatment. There seems to be a significant between-patient variation in cell yield and expansion time. A previous study has demonstrated that kidney function, chronic obstructive pulmonary disease, and use of steroids are related to the number of mesenchymal stromal cells (MSCs) reached after culture expansion [10]. In addition, intervention with fresh cells faces great logistic challenges such as transportation and timing of treatment. To minimize donor variability and the influence of patient comorbidities on product quality, we have shifted focus from autologous to allogeneic ASC therapy by using young and healthy volunteer donors of adipose tissue. The use of allogeneic MSCs is perceived viable because these cells are immune evasive. They have both regenerative and immunomodulatory properties—most intriguingly immunosuppressive ones [11–13]. As such, it is rendered probable that these cells evade being recognized by a recipient immune system, therefore allowing allogeneic use. Allogeneic MSCs and ASCs have already been used in clinical trials without any side effects [14–16].

With an eye on feasibility during manufacturing, standardization, and product reproducibility, we have implemented production in semi-automated closed bioreactor systems instead of manual handling of flasks and also the use of human platelet lysate instead of fetal bovine serum as a growth supplement [17–19]. Finally, we have abandoned fresh cell delivery and have developed a cryopreserved off-the-shelf product to improve clinical applicability.

The aim of the present phase I safety study was to test the safety profile and feasibility of our newly developed, cryopreserved, and allogeneic ASC product from healthy donors in patients with IHF.

Materials and Methods

Study Design

A single-center first-in-human phase I study to investigate the safety and efficacy of direct intramyocardial injections of the Cardiology Stem Cell Centre adipose-derived stromal cell product (CSCC_ASC) in ten patients with chronic IHF.

The study protocol complies with the Declaration of Helsinki and was approved by the Danish National Committee on Health Research Ethics (No. 1404435) and Danish Medicines Agency (Eudra-CT: 2014-002980-13). The study is registered at clinicalTrials.gov (NCT02387723). The local Good Clinical Practice Unit monitored the study.

Study Population

The study included patients between 30 and 80 years of age with chronic IHF, reduced left ventricular ejection fraction (LVEF; $\leq$45%), New York Heart Association (NYHA) class II–III, no further revascularization options, and on maximal tolerable medical therapy. The patients were all enrolled and treated at Rigshospitalet University Hospital Copenhagen. Inclusion and exclusion criteria are described in Supporting Information Appendix 1.

Study Procedures and Timeline

There was at least 1 week between treatments of each of the first three patients in order to observe and evaluate safety of the therapy. Thereafter, the remaining seven patients were treated. All patients stayed in-hospital at least 1 day after treatment. Patient follow-up visits were done after 1, 2, 3, and 6 months, and patients were evaluated clinically, with blood analyses for hemoglobin, leukocytes, platelets, sodium, potassium, creatinine, high-sensitivity CRP (hs-CRP), creatine kinase MB (CKMB), troponin, and human leukocyte antigen (HLA) antibodies.

At baseline, and at 3- and 6-month follow-up, 6-minute walking tests (6MWT), Canadian Cardiovascular Society (CCS) and New York Heart Association (NYHA) classifications, and Kansas City Cardiomyopathy Questionnaires (KCCQ) were performed. Cardiac computed tomography (CT) scans or echocardiography (ECHO) were performed at baseline and 6 months after treatment.

Donors

Lipoaspirates were obtained from three healthy female donors (28–33 years old). Donor eligibility was determined by a donor interview, a questionnaire, and testing for infectious disease markers HIV, hepatitis B and C, and syphilis by serum analyses within 30 days prior to liposuction. In addition, a blood sample was drawn on the day of donation for repeated serology and nucleic acid testing of HIV, and hepatitis B and C. Donor testing was performed by the Virus Laboratory, The Blood Bank, Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, as authorized by The Danish Patient Safety Authority. The use of lipoaspirate has been approved by competent authorities as mentioned above. All donors signed an informed consent.

Production of CSCC_ASC

Liposuction was performed from abdominal adipose tissue by an experienced plastic surgeon, with local anesthesia, and in full compliance with surgical procedures for sterile cosmetic surgery. Liposuction was performed at the Department of Plastic Surgery, Breast Surgery and Burns, Rigshospitalet and at Printzlau Private Hospital, Virum, Denmark. Between 100 and 150 mL of abdominal adipose tissue was obtained from each donor.

Preparation of lipoaspirates, isolation of stromal vascular fractions (SVF), and expansion of cells in Quantum Cell Expansion Systems was performed at Cardiology Stem Cell Centre, as previously described [17–19]. In short, SVF was isolated by enzymatic digestion from each lipoaspirate, and expansion of cells was performed in a two-passage expansion process in the semi-automated Quantum Cell Expansion System. Cell culture medium was Minimum Essential Medium, MEM Alpha (αMEM) without ribonucleosides and deoxyribonucleosides, (Gibco, Gaithersburg, MD, https://www.thermofisher.com/us/en/home/brands/gibco.html; Life Technologies, Durham, NC, https://www.thermofisher.com/us/en/home.html), 1% penicillin/streptomycin (100 U/mL and 100 μg/mL, respectively; Gibco, Life Technologies) and 5% Stemulate (human platelet lysate, Cook Regentec, Indianapolis, IN, http://www.cookregentec.com/). For the purpose of cryo storage as a ready-to-use product, the final cell product constituting 110 million ASCs in 5 mL cryopreservation media (CryoStor10, Biolife

Table 1. Demographic data of the 10 patients with ischemic heart failure

Parameters	*n* = 10, *n* (%)
Age, years ± SD	62.5 ± 6.6
Gender, male	7 (70)
BMI, kg/m^2 ± SD	30.2 ± 6.7
LVEF, % ± SD	28.8 ± 4.1
Smoking	
Current	2 (20)
Previous	4 (40)
Never	4 (40)
Diabetes mellitus	3 (30)
Hypertension	5 (50)
Hypercholesterolemia	9 (90)
Previous AMI	10 (100)
Previous CABG	4 (40)
Previous PCI	7 (70)
Systolic blood pressure, mmHg ± SD	123 ± 15
Diastolic blood pressure, mmHg ± SD	73 ± 10
Pro-BNP, pmol/L ± SD	84 ± 61
6MWT, meter ± SD	435 ± 87
Treatment with	
Beta-blocker	9 (90)
ACE-inhibitor	9 (90)
Diuretic	6 (60)
Nitrate	4 (40)
Statin	9 (90)

Abbreviations: 6MWT, 6-minute walking test; ACE-inhibitors, angiotensin-converting-enzyme inhibitor; AMI, acute myocardial infarction; BMI, body mass index; CABG, coronary artery by-pass grafting; LVEF, left ventricular ejection fraction; *n*, number of patients; PCI, percutaneous voronary intervention; pro-BNP, pro-brain natriuretic peptide.

Solutions, Bothell, WA, http://www.biolifesolutions.com/) was portioned into CellSeal vials (Cook Regentec).

Freezing was performed with a controlled rate freezer, Kryo 560–16 (Planer PLC, Sunbury-on-Thames, United Kingdom, https://planer.com/). After freezing, vials were stored in a CBS-v1500 Isothermal all dry-storage system (Custom Biogenic Systems, Bruce Township, MI, http://www.custombiogenics.com/) below −180°C.

Preparing for treatment, CellSeal vials were thawed in a 37°C water bath. Cell suspensions were aspirated with a needle into a sterile syringe. The syringe was subsequently connected to the MYOSTAR injection catheter (Biological Delivery System, Cordis, Hialeah, FL, https://www.cordis.com/global-home.html; Johnson & Johnson, New Brunswick, NJ, https://www.jnj.com/) for injection. Injection was performed within 1 hour of thawing.

Quality Control

CSCC_ASC was released for clinical use based on cell number, viability, donor serology, sterility, and ASC characterization according to the International Society for Cellular Therapy (ISCT) and the International Federation for Adipose Therapeutics and Science (IFATS) criteria (Supporting Information Appendix 2) [18, 19].

Table 2. Serious adverse events and adverse events within the 6-month follow-up period in 10 patients after treatment with Cardiology Stem Cell Centre adipose-derived stromal cell

Serious adverse events	*n* = 3	Patient no.
Death	1	6
Hospitalizations		
Unstable angina pectoris	1	3
Dyspnea	1	3
Adverse events	***n* = 9**	**Patient no.**
Pneumonia	1	3
Deterioration heart failure	3	1, 3, 5
Herpes Zoster	2	5, 8
Bleeding from rectum–colonoscopy normal	1	5
Febrile–virus	1	1
Insomnia problems	1	10
Deterioration of treated depression	1	3

Total number of cells and percentage viability was determined with a NucleoCounter NC-100 (Chemometec, Allerød, Denmark, https://chemometec.com/) based on detection of fluorescence from the DNA binding dye, propidium iodide. Microbiological quality control on final cell product was performed with a fully validated protocol using aerobic and anaerobic BacT/ALERT iFN and iFA plus culture bottles (Biomerieux, Durham, NC, http://www.biomerieux-usa.com/) and the BacT/ALERT Microbial Detection System (Biomerieux). Presence of mycoplasmas in cell culture supernatants was detected by polymerase chain reaction (PCR) for mycoplasma genus DNA at Statens Serum Institute, Copenhagen, Denmark. The content of endotoxins was quantitatively determined by the limulus amebocyte lysate chromogenic endpoint method, by Statens Serum Institute. Immunophenotyping of ASCs was performed by flow cytometry according to ISCT and IFATS standards [20, 21].

The following markers were used for release criteria (Supporting Information Appendix 2): cluster of differentiation (CD)45-fluorescein isothiocyanate (FITC), HLA-DR-FITC, CD90-FITC, CD73-phycoerythrin (PE) (all Beckman Coulter, Pasadena, CA, https://www.beckmancoulter.com/), and CD105-PE (R&D Sciences, Denmark). An extended panel of ISCT/IFATS markers was used for research purpose. A compensated six-color protocol including isotypic controls and Fluorescence Minus One tubes was used (Navios flow cytometer, Beckman Coulter). Viability was determined with SYTOX blue (SYTOX, Invitrogen, Carlsbad, CA, https://www.thermofisher.com/us/en/home/brands/invitrogen.html; Life Technologies). Dead cells were excluded from the final analysis and data were analyzed using Navios software and Kaluza (Beckman Coulter).

Genomic stability as determined by comparing ASCs at initial and final passage with comparative genomic hybridization (CGH) was performed by Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital. CGH was performed using the Agilent SurePrint G3 Human CGH Microarray kit 8_60K (design ID 021924) with 41 Kb overall median probe spacing (Agilent Technologies, Cary, NC, http://www.agilent.com/). Arrays were analyzed using an Agilent SureScan Microarray scanner and the Agilent Feature Extraction software (v11.5), and results were presented by Agilent Genomic Workbench (v.7.0).

Table 3. Development of donor HLA-specific antibodies

Donor	HLA typing	Patient	Allele	Donor-specific HLA antibodies (MFI)				
				Baseline	1 month	2 months	3 months	6 months
1	A*01; B*08; C*07	1		(-)	(-)	(-)	(-)	(-)
	DRB1*03; DQB1*02	2		(-)	(-)	(-)	(-)	(-)
		3		(-)	(-)	(-)	(-)	(-)
		4	B*08	(-)	(-)	(-)	2000	N/A[a]
2	A*02; B*07,*57; C*06,*07;	5	B*57	(-)	4000	2000	1500	(-)
	DRB1*07,*15; DQB1*03,*06	6	A*02	(-)	2000	3000	4000	2000
			B*07	(-)	(-)	(-)	(-)	2000
			B*57	(-)	6000	7000	8000	3000
		7	B*07	2500	4000	3500	3500	1500
			DR15	6500	10500	11500	11000	7000
		8	A*02	13000	15000	12000	16500	4000
			B*07	8000	16000	13000	15000	6000
			B*57	(-)	11000	16500	10100	7500
3	HLA-A*02,*B40,*44; C*02,*05;	9	A*02	(-)	(-)	(-)	1000	2500
	DRB1*04,*13;DQB1*03,*06	10		(-)	(-)	(-)	(-)	(-)

Serum alloantibodies matching donor genotypes were evaluated at fixed time points spanning 6 months. To illustrate titers semiquantitatively, MFI is listed for detected allele.
[a]Patient passed away (unrelated cause). (-): MFI $<$ 1,000.
Abbreviations: HLA, human leukocyte antigen; MFI, median fluorescence intensity.

Table 4. Changes in cardiac function, exercise capacity, and symptoms from baseline to 6 months after direct intramyocardial injections of Cardiology Stem Cell Centre adipose-derived stromal cell

Functional parameters	*n*	Baseline	6-month follow-up	Difference	SD	95% confidence interval		*p* value[a]
						Lower	Upper	
LVESV	9	205 mL	182 mL	−23 mL	34	−3	49	.073
LVEF	9	28.8%	31.7%	2.9%	4.1	0.2	6.1	.065
LVEDV	9	285 mL	279 mL	6 mL	26	−14	26	.503
6MWT	8	460 m	495 m	35 m	14	24	47	$<$.0001
KCCQ QoL	9	67	65	1.9	17.6	−12	15	.760
NYHA	10	2.8	2.2	0.6	0.8	0	1.2	.063
CSS	10	0.8	0.7	0.1	0.7	−0.4	0.6	.655

[a]*p* value between groups for differences.
Abbreviations: 6MWT, 6-minute walking test; CCS, Canadian Cardiovascular Society angina classification; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEDV, left ventricle end-diastolic volume; LVEF, left ventricle ejection fraction; LVESV, left ventricle end-systolic volume; m, minutes; NYHA, New York Heart Association classification; QoL, quality of life.

The residual amount of penicillin has been measured by liquid chromatography-mass spectrometry in representative samples of treatment vials during development. Due to a thorough washing procedure, the amount of residual penicillin is found to be of no risk with regard to causing allergic reactions; amounts are lower than first doses used for intravenous desensitization protocols [22].

HLA Tissue Typing and of HLA Tissue Antibodies Measurements

All donors had an intermediate resolution typing of HLAs (HLA)-A, B, C, DRB1, DRB345, DQA1, DQB1, DPA1, DPB1 loci by real-time PCR with subsequent melting point analyses using a Linkseq 384-well complete typing kit (Linkage Biosciences, San Francisco, CA, https://www.linkagebio.com/). The patients had a measurement of anti-HLA antibodies in serum before as well as 1, 2, 3 and 6 months after the treatment. The LABScreen HLA class I and II single antigen bead assay on a Luminex 100 (One Lambda, Inc., Thermo Fisher, Canoga Park, CA, http://www.onelambda.com/) was used. Tests were performed in accordance with the manufacturer's instructions and laboratory standard operating procedures for clinical samples. Trimmed mean values were normalized for background and expressed as mean fluorescence intensity (MFI). Cut-off for positivity was defined as MFI $\geq$1.000. The laboratory performance of the analyses was accredited by the European Federation of Immunogenetics.

NOGA-Guided Injection

A 3D electromechanical mapping of the left ventricle endocardium was created with the NOGA system (Biologics Delivery

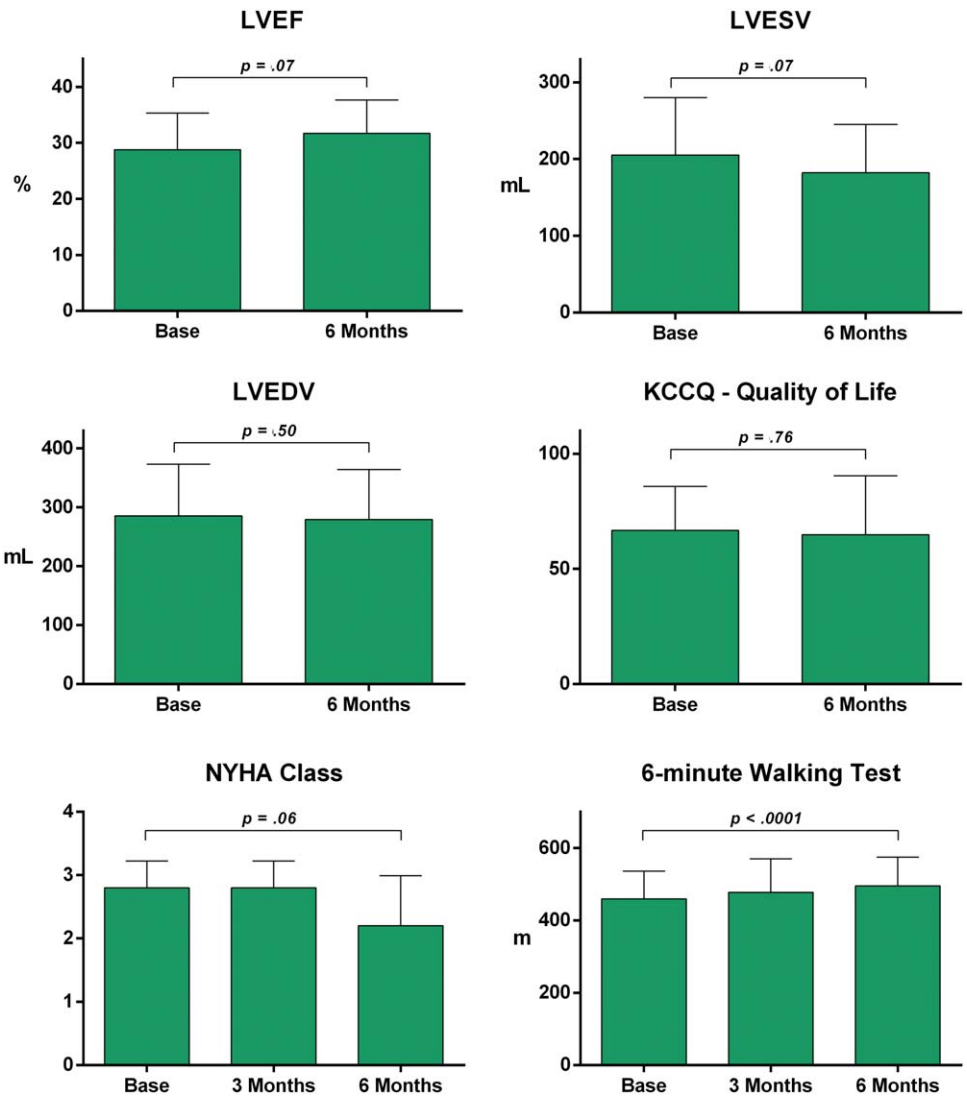

Figure 1. Cardiac function, exercise capacity and symptoms before and 6 months after treatment with Cardiology Stem Cell Centre adipose-derived stromal cell. Mean ± SD. Abbreviations: KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEDV, left ventricle end-diastolic volume; LVEF, left ventricle ejection fraction; LVESV, left ventricle end-systolic volume; NYHA, New York Heart Association classification.

Systems, CA, Cordis Corporation, Johnson & Johnson Company, US) using a NOGA Myostar catheter (Biological Delivery System). Approximately 15 injections of 0.3 mL CSCC_ASC (total 100 million) were injected via transendocardial stem cell injection into viable myocardium in the border zone of infarcted tissue.

Endpoints

The primary endpoint was major immunologic reaction of allogeneic CSCC_ASC therapy with respect to incidence and severity of serious adverse events and suspected unrelated serious adverse events. To be open for all potential serious events, we had not, in the protocol, specified events in details. However, we were looking for immunologic reactions, death, hospitalization for worsening heart failure including inserting of a biventricular pacemaker, and hospitalization due to ventricular tachycardia or fibrillation, cardiac perforation, and pericardial tamponade.

The secondary endpoints were improvements in left ventricle end-systolic volume (LVESV), LVEF, and left ventricle end-diastolic volume (LVEDV) at 6-month follow-up. Other secondary endpoints were NYHA classification, CCS angina classification, KCCQ, 6MWT, and development of donor antibodies at 6-month follow-up.

Cardiac CT Acquisition and Analysis

A 320-multidetector CT scanner (Aquilion One, Toshiba Medical Systems Corporation, Otawara, Japan, http://www.toshibamedicalsystems.com/) was used to perform a cardiac CT scan before and 6 months after therapy, as described previously [8, 23, 24]. The R-R interval and multisegmental image reconstruction was performed with the scanner software. Images were reconstructed with 0.5 mm slice thickness and increments of 0.25 mm in 2% interval in the prospective window.

Participants underwent a standard transthoracic echocardiographic examination using Philips cardiovascular ultrasound system with a X5–1 probe (Philips, Cardiovascular, US) for standard ECHO. Images were stored for offline analysis.

All image data was analyzed with the CVI42 post-processing tool (Circle Cardiovascular Imaging, Calgary, Alberta, Canada, https://www.circlecvi.com/). Endocardial and epicardial borders

were traced manually in end-diastole and end-systole, and the mitral plane set to define the basal border of the left ventricle.

Statistics

Analyzes were performed using the statistical software SPSS version 23.0 (SPSS Inc., Chicago, Illinois, http://www.ibm.com/analytics/us/en/technology/spss/). Continuous variables are presented as mean ± standard deviation and categorical variables are presented as numbers and percentages. Within group comparison were analyzed using paired *t* test for continuous data and Wilcoxon signed ranks test for categorical data. Between groups comparisons were analyzed with Student's *t* test. A two-tailed probability value less than .05 was considered to indicate statistical significance.

Results

Patients

A total of ten patients (seven men and three women; mean age: 62.5 ± 6.6 years) with stable IHF were included in the study. Demographic data are presented in Table 1.

Cell Characteristic

Immunophenotype. CSCC_ASC phenotype was in accordance with ISCT and the Joint statement of the IFATS [20, 21]. Release criteria are shown in Supporting Information Appendix 2.

Genomic Stability. Array CGH analysis of cells from the donors demonstrated that ASCs expanded in vitro in Quantum Cell Expansion devices in the presence of humane platelet lysate did not show imbalanced chromosomal rearrangements.

Microbial Tests. Microbial tests for aerobic and anaerobic bacteria, fungus of final cell preparations, and mycoplasmas from culture supernatants were all negative. The endotoxin concentration in all final cell preparations was less than 10 IU/mL.

Safety

Each patient was treated with cells from one of three donors, and no matching between the donor and the patient tissue types was performed. There were no procedure-related complications to the direct intramyocardial injection of CSCC_ASC.

One patient died 6 months after treatment during a hospitalization for a knee bacterial bursitis with cardiac complications. This event was not related to the stem cell treatment. One patient had two hospitalizations due to unstable angina pectoris and dyspnoea, respectively. Coronary angiography was without any new lesions, and diuretic treatment was intensified (Table 2).

During the 6-month follow-up period, four out of ten patients developed donor-specific de novo HLA class I antibodies, and two other patients had donor-specific antibodies at baseline (Table 3). None of the patients had any clinical symptoms or changes in biochemical parameters (leukocytes, high-sensitive CRP) or inflammatory signs indicating an immunization.

An expected increase and decrease in troponins and CKMB, respectively, after the intramyocardial injection of cells was seen (data not shown). There was no increase in hemoglobin, leukocytes, platelets, sodium, potassium, creatinine, hs-CRP, CKMB, and troponin in the follow-up period (data not shown).

Table 5. Influence of development of donor specific antibodies on changes in cardiac function, exercise capacity and symptoms from baseline to 6 months after direct intramyocardial injections of Cardiology Stem Cell Centre adipose-derived stromal cell

Parameter	Difference		*p* value[a]
	No antibodies (*n* = 6)	Antibodies (*n* = 3)	
LVESV (mL)	−16.5 ± 33.6	−36.2 ± 36.0	.44
LVEF (%)	3.6 ± 4.0	1.6 ± 4.7	.52
LVEDV (mL)	−6.8 ± 32.4	−4.5 ± 4.1	.91
6MWT (m)	35.9 ± 15.5	33.5 ± 9.2	.85
KCQoL	−1.7 ± 19.9	−2.1 ± 17.2	.97
NYHA	−0.7 ± 0.8	−0.5 ± 1.0	.78

All values are mean differences + standard deviation.
[a]*p* value between groups for differences.
Abbreviations: 6MWT, 6-minute walking test; CCS, Canadian Cardiovascular Society angina classification; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEDV, left ventricle end-diastolic volume; LVEF, left ventricle ejection fraction; LVESV, left ventricle end-systolic volume; m, minutes; NYHA, New York Heart Association classification.

Efficacy

The cardiac function tended to improve after CSCC_ASC treatment at 6-month follow-up: LVESV decreased from 205 mL to 182 mL with a difference of 23 mL (95% confidence interval [CI]: −3 to 49; $p = .073$), and LVEF increased from 28.8% to 31.7%, with a difference of 2.9% (95% CI: 0.2 to 6.1; $p = .065$). In addition, 6MWT increased from 460 minutes to 495 minutes, with a difference of 35 minutes (95% CI: 24 to 47; $p < .0001$), and NYHA class from 2.8 to 2.2, with a difference of 0.6 (95% CI: 0 to 1.2; $p = .06$) 6 months after therapy (Table 4; Fig. 1).

There were no differences in KKCQ scores and CCS class in the follow-up period (Table 4). Plasma pro-brain natriuretic peptide was unchanged from baseline (84 ± 61 pmol/L) to 6-month follow-up (87.8 ± 23.9 pmol/L; $p = .38$).

The development of tissue type-specific donor antibodies seemed to have no negative effect on cardiac function, exercise capacity, and symptoms 6 months after treatment with CSCC_ASC (Table 5; Fig. 2).

Discussion

The present study is the first-in-human study of a newly developed cryopreserved off-the-shelf adipose-derived stromal cell product (CSCC_ASC), developed from healthy donors without the use of xenogeneic animal constituents while using a closed bioreactor system for culture expansion of cells. A total of 110 million ASCs were injected directly into the myocardium with no complications or serious adverse events related to either treatment or cell administration.

No pretreatment tissue type matchings between the donors and the patients were carried out. Although two patients had donor-specific HLA antibodies at baseline and four patients developed donor-specific de novo HLA class I antibodies after treatment, this seemed to have no influence on the efficacy of the cell product in patients with IHD and heart failure. De novo antibodies were only directed against HLA class I antigens, as expected, because CSCC_ASC does not present HLA class II antigens.

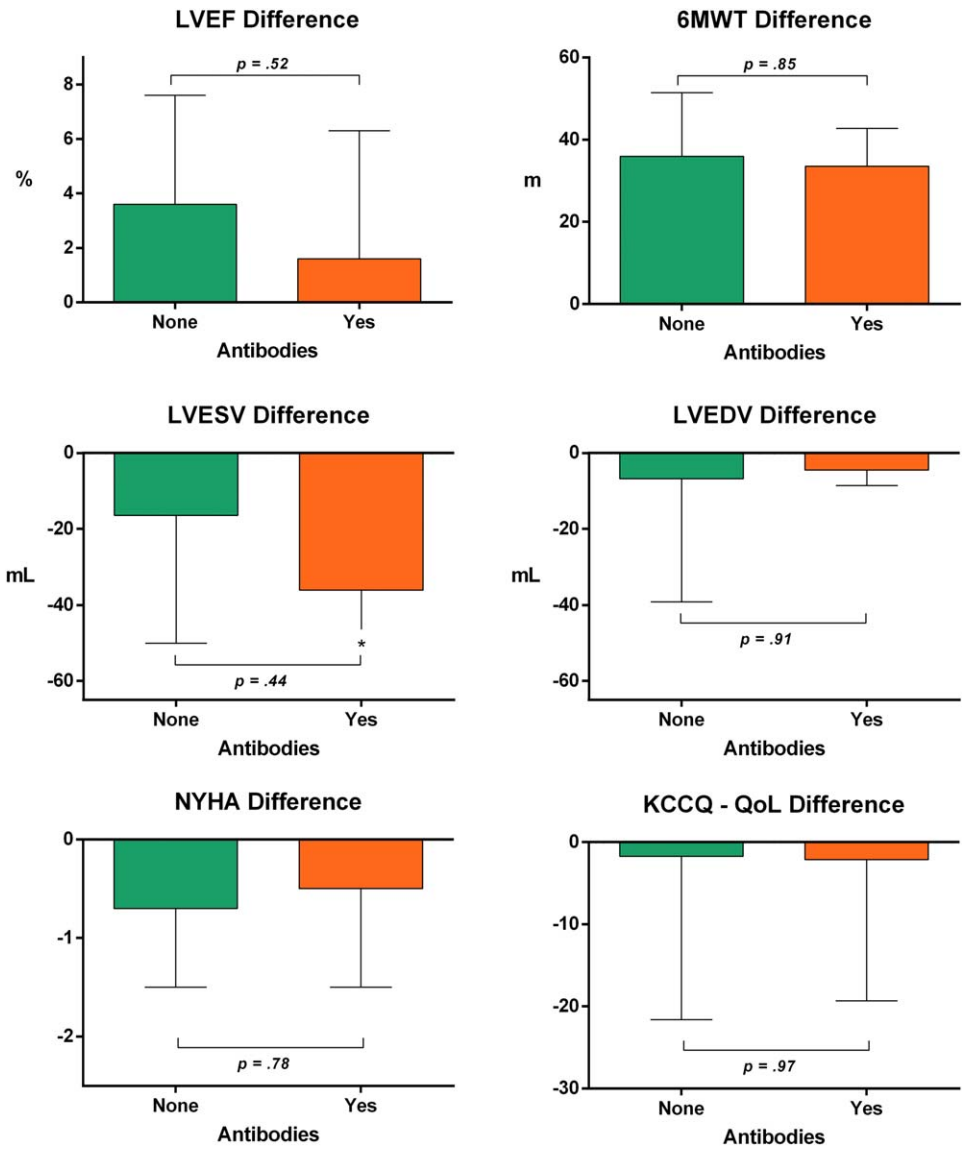

Figure 2. The influence of development of donor-specific tissue type antibodies on cardiac function, exercise capacity, and symptoms before and 6 months after treatment with Cardiology Stem Cell Centre adipose-derived stromal cell. Mean ± SD. The * in LVESV indicates that the SD is outside the lower border of the figure. Abbreviations: 6MWT, 6-minute walking test; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEDV, left ventricle end-diastolic volume; LVEF, left ventricle ejection fraction; LVESV, left ventricle end-systolic volume; NYHA, New York Heart Association classification; QoL, quality of life.

It is a limitation that HLA antibodies were measured continuously for each patient during the study period, as this does not allow direct comparisons of MFI values between samples for each patient due to day-to day variation in the laboratory.

Although not powered to demonstrate any efficacy, the CSCC_ASC treatment demonstrated a tendency toward an improvement in left ventricle pump function and a reduction in dilatation of the left ventricle. Although these findings have to be confirmed in a larger clinical trial, they display the same tendency that we have previously demonstrated in a larger double-blind placebo-controlled study in patients with heart failure treated with autologous BMSCs. [8] In addition, the present study found a significant improvement in 6MWT.

As demonstrated for autologous cell therapy, the findings strongly support the hypothesis that allogeneic mesenchymal stromal cells are safe and efficacious. Moreover, there seems to be no need for immunosuppressive treatment concomitant with the allogeneic cell therapy. The present study extends the previous demonstration of safety using the autologous adipose-derived SVF treatment of patients with chronic ischemic cardiomyopathy (Precise Trial), non-ischemic dilated cardiomyopathy (POSEIDON-DCM Trial), and patients with acute myocardial infarction (Apollo Trial) [25–27].

The overall aim was to establish a logistically and clinically applicable off-the shelf stem cell treatment strategy for patients. We changed from autologous to allogeneic cells, and we implemented a more standardized and reproducible stem cell production platform using semi-automated closed bioreactor systems instead of flasks and human platelet lysate instead of fetal bovine serum, as well as cryo storage of a ready-to-use product [7–19].

We have in the present study demonstrated that it is feasible to generate a reproducible, standardized, and clinically applicable cell product from healthy donors that is safe and has a very promising efficacy profile.

One obvious consequence of using a cryopreserved cell product for clinical treatment is the use of cryopreservation formulas holding dimethyl sulfoxide (DMSO). Clinical side effects have been related to the amount of DMSO administered; however, with given intramyocardial administration, a total amount of only 0.5 g DMSO, distributed in 15 injections, does not give rise to any side effects. This is in accordance with previous publications in which intramyocardial and intravenous injection of cells in excipients holding DMSO were proven safe in clinical studies for heart diseases [27–29].

It is a common strategy for the sake of standardization and optimization of efficacy to produce allogeneic mesenchymal stromal cell products from one donor only. However, this limits dissemination when a new donor eventually has to be included due to increased scale of treatments and the inevitable senescence of the original stromal cell line. We have used cell product from three donors, which induces a higher degree of variability in cell products and potentially also in clinical efficacy. However, it also extends the safety profile and the efficacy to a more general use of donors, which can be a benefit in the long run, after implementation of the therapy as a more general treatment option.

Conclusion

The present study was a phase I study with no control group. Therefore, some of the efficacy findings are potentially due to a placebo effect. Also, the study was not powered to evaluate efficacy, and statistical results should be viewed with this in mind. To further evaluate the safety and efficacy of this newly developed allogeneic ASC product, larger double-blind placebo-controlled clinical trials are needed in patients with IHF.

In conclusion, the present first-in-human study of the newly developed allogeneic ASC product CSCC_ASC from healthy donors stored frozen as an off-the-shelf product demonstrated safety and feasibility when injected directly into the myocardium in patients with IHF.

Author Contributions

J.K.: conception and design, provision of study material from healthy donors, patient recruitment and treatment, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; M.H.-S., M.J., R.H.S., B.F., L.D.L., A.E.: isolation, production, storage, quality control, characterization of the new drug CSCC_ASC, final approval of manuscript; J.J.E.: liposuction of donors, final approval of manuscript; E.J., S.H.: treatment of patients, final approval of manuscript; A.B.M., A.A.Q.: imaging and data analysis, final approval of manuscript; H.B.: tissue typing and antibodies analyses, final approval of manuscript; E.M.J.: final approval of manuscript.

Disclosures of Potential Conflicts of Interest

J.K., A.E., and M.H.S. have filed an International (PCT) patent application No. PCT/EP2016/075407 "Stem cell therapy in patients with ischenmic heart disease". H.B. has received honoraria for a lecture. The other authors indicated no potential conflicts of interest.

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