CLINICAL TRACK

Safety and Efficacy of Intracoronary Infusion of Allogeneic Human Cardiac Stem Cells in Patients with ST-segment Elevation Myocardial Infarction and Left Ventricular Dysfunction: A Multicenter Randomized, Double-Blind and Placebo-Controlled Clinical Trial

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ABSTRACT

***Rationale*:** Allogeneic cardiac stem cells (AlloCSC-01) have shown protective, immunoregulatory and regenerative properties with a robust safety profile in large animal models of heart disease.

***Objective*:** To investigate the safety and feasibility of early administration of AlloCSC-01 in patients with ST-segment elevation myocardial infarction (STEMI).

***Methods and Results*:** CAREMI was a phase I/II multicenter, randomized, double-blind, placebo-controlled trial in patients with STEMI, LVEF ≤ 45% and infarct size ≥ 25% of left ventricular (LV) mass by cardiac magnetic resonance (MR), who were randomized (2:1) to receive AlloCSC-01 or placebo through the intracoronary route at day 5-7. The primary endpoint was safety and included all-cause death and major adverse cardiac events at 30 days (MACE: all-cause death, reinfarction, hospitalization due to heart failure, sustained ventricular tachycardia, ventricular fibrillation and stroke). Secondary safety endpoints included MACE at 6 and 12 months, adverse events (AE) and immunological surveillance. Secondary exploratory efficacy endpoints were changes in infarct size (% LV mass) and indices of ventricular remodeling by MR at 12 months. Forty-nine patients were included (92% male, 55±11 years), 33 randomized to AlloCSC-01 and 16 to placebo. No deaths or MACE were reported at 12 months. One severe AE in each group was considered possibly related to study treatment (allergic dermatitis and rash). AlloCSC-01 elicited low levels of donor specific antibodies in 2 patients. No immune-related AE were found and no differences between groups were observed in MR-based efficacy parameters at 12 months. The estimated treatment effect of AlloCSC-01 on the absolute change from baseline in infarct size was -2.3% (95%CI -6.5 to 1.9%).

***Conclusions*:** Allogeneic cardiac stem cells can be safely administered in STEMI patients with LV dysfunction early after revascularization. Low immunogenicity and absence of immune-mediated events will facilitate adequately powered studies to demonstrate their clinical efficacy in this setting.

Clinical Trial Registration: NCT 02439398.

Keywords:
Acute myocardial infarction, heart failure, allogeneic stem cell therapy, cardiac stem cells, cardiac remodeling.

Nonstandard Abbreviations and Acronyms:

AE	adverse event.
AlloCSC-01	allogeneic cardiac stem cells.
CABG	coronary artery bypass grafting.
CAD	coronary artery disease.
CI	confidence interval.
CDC	cardiosphere-derived cells.
CK	creatine kinase.
CRP	C-reactive protein.
CSC	cardiac stem cells.
DSA	donor specific antibody.
DSMB	data safety monitoring board.
ECG	electrocardiography.
EMA	European Medicines Agency.
FAS	full analysis set.
HF	heart failure.
HLA	human leukocyte antigen.
IQ	interquartile range.
LAD	left anterior descending coronary artery.
LV	left ventricle.
LVEF	left ventricular ejection fraction.
MACE	major adverse cardiac events.
MLHFQ	Minnesota Living with Heart Failure Questionnaire.
MR	magnetic resonance.
MSC	mesenchymal stem cells.
NT-proBNP	N-terminal pro-brain natriuretic peptide.
NYHA	New York Heart Association.
PCI	percutaneous coronary intervention.
PPS	per protocol set.
qPCR	quantitative polymerase chain reaction.
SAE	serious adverse event.
SAS	safety analysis set
STEMI	ST-segment elevation myocardial infarction.
TIMI:	“Thrombolysis In Myocardial Infarction” flow grade.

INTRODUCTION

Pharmacological and interventional therapies have dramatically reduced the mortality of ST-segment elevation myocardial infarction (STEMI), but have paradoxically contributed to an increased incidence of post-infarction chronic heart failure (HF), which affects between 6% and 25% of survivors at 5 years depending on age and ethnicity.[1]

Cardiac stem cells (CSC) have been investigated in the treatment of STEMI to prevent adverse left ventricular (LV) remodeling and the development of chronic HF. Preclinical evidence indicates that these cells produce and locally secrete a multitude of protein-based factors with immunoregulatory properties. CSC may protect cardiomyocytes and other cells in the zone at risk of dying during recovery from ischemia, promote angiogenesis, reduce scar formation and activate endogenous CSC.[2, 3] In concert, these mechanisms are thought to limit subsequent ventricular damage, prevent the LV adverse remodeling process and ultimately confer functional benefits.[4]

CSC of autologous origin show variable functionality depending on the age, comorbidities and genetic signatures of the host.[5] Some of these limitations can be overcome using allogeneic sources from healthy donors, thereby improving the biological potential of regenerative therapy.[6] In addition, allogeneic cells can be manufactured in large quantities, which opens new treatment opportunities during the acute stages of STEMI, at the critical time window of healing and scar formation.

The "Safety and Efficacy of Intracoronary Infusion of Allogeneic Human Cardiac Stem Cells in Patients with STEMI and Left Ventricular Dysfunction" (CAREMI) study was a phase I/II, randomized, double-blind, placebo-controlled clinical trial. A suspension of allogeneic human CSC (AlloCSC-01) was injected in patients with a first STEMI through the intracoronary route during the acute phase. This trial was designed as a two-phase study consisting of an open-label dose-escalation phase followed by a double-blind randomized phase. Primary endpoints were safety and feasibility of intracoronary allogeneic CSC infusion in STEMI and secondary efficacy endpoints included magnetic resonance (MR) imaging-based measurements of infarct size and LV remodeling.[7, 8]

METHODS

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study design and oversight.

The design of this clinical trial has been reported previously.[9] Study data were collected with an electronic-record form and were managed by Chiltern International Spain S.A. (Madrid, Spain). Consolidated data were transferred to KU Leuven Research and Development (LCC, Leuven, Belgium) for independent statistical analysis. The trial was approved by national regulatory authorities and by local ethics committees at all participating centers, and all patients provided written informed consent.

Patients.

Patients were eligible for enrollment if they were between 18 and 80 years old, presented with STEMI and had a medium-high risk of developing chronic HF. The latter was defined as LVEF $\leq$ 45% and infarct size $\geq$ 25% of LV mass in the screening MR scan performed 3 to 5 days after STEMI onset. Successful revascularization by percutaneous coronary intervention (PCI), within 12h after symptom onset with stent implantation and Thrombolysis in Myocardial Infarction (TIMI) flow grade 3, had to be achieved. All critical, non-infarct-related artery lesions should have been treated percutaneously at least 24 hours before MR evaluation. Patients were recruited in eight participating tertiary academic hospitals.

Therapeutic product.
AlloCSC-01 is a suspension of allogeneic human CSC in saline solution with 5% human serum albumin (total volume = 18 mL). CSC were isolated from human heart biopsies (right atrial appendages), donated by three patients undergoing cardiac surgery (usually valve replacement procedures) after providing informed consent. Tissue samples were minced into small pieces and treated with collagenase type 2 (Worthington Biochemical Corporation, Lakewood, NL, USA). CSC were obtained after immunodepletion of $CD45^+$ cells and immunoselection of $CD117^+$ ($c\text{-}kit^+$) cells, using specific microbeads (Miltenyi Biotech, Bergish, Glabach, Germany), and finally expanded (five passages) at a certified Good Manufacturing Practice facility (3P Biopharmaceuticals, Noáin, Spain). During the manufacturing process, AlloCSC-01 complied with safety and quality standards (ie, number of population doublings, doubling times, genomic stability by comparative genomic hybridization and sterility testing), which were assessed in each batch according to the European Medicines Agency (EMA) Guidelines. Phenotypic and functional characterizations of the cells were done in all three final cell batches to ensure bioequivalence before initiating the trial. Placebo consisted of 18 mL of human serum albumin 5% in saline solution. For a detailed methodological description of AlloCSC-01 characterization and quality assurance see Online Supplement.

Intracoronary delivery of study product (AlloCSC-01 or placebo) in the culprit vessel was performed 5 to 7 days after reperfusion using an over-the-wire coronary perfusion catheter (Progreat, Terumo Interventional Systems, Tokyo, Japan). The study product was delivered in three consecutive boluses of 6 mL by manual infusion over 3 minutes, with 3 minute intervals between boluses. Each bolus was preceded by nitroglycerin (200-400 μg) and followed by 1 mL of saline to flush the catheter delivery system.

Randomization and blinding.
In the dose-escalation phase all patients received active treatment at increasing doses of AlloCSC-01. The target dose was $35x10^6$ cells, based on preclinical experiments.[10] The first dose to be tested was $10x10^6$ cells and the dose escalation process comprised 3 steps ($10x10^6$, $20x10^6$ and $35x10^6$) with a primary follow-up of 7 days for each group of 2 patients before the next escalation step was initiated.
Once the dose-escalation short-term safety data was analyzed by the Data and Safety Monitoring Board (DSMB), patients were randomly allocated 2:1 to receive AlloCSC-01 ($35x10^6$) or placebo. Assignment was performed by an automated interactive voice-response system in random blocks of 3. The presence of CSC in the final product was masked for the interventional team with translucent sterile adhesive films around the syringe. Patients and all evaluators were blinded to treatment assignment.

Endpoints.
The primary endpoint of the trial was the combination of all-cause death and major adverse cardiac events at 1 month (MACE: all-cause death, reinfarction, hospitalization due to HF, sustained ventricular tachycardia, ventricular fibrillation and stroke). Secondary safety endpoints included all-cause death, MACE and all-cause adverse events (AE) at 6 and 12 months, and immunological surveillance by human leukocyte antigen (HLA) typing, cross-matching between cells/patient serum and Luminex-based identification of anti-HLA class I and class II antibodies (immunoglobulin G) pre/post-treatment. The main secondary efficacy endpoint was MR-based infarct size. Additional exploratory secondary efficacy endpoints were based on MR variables (indices of ventricular remodeling and function), laboratory (natriuretic peptides, C-reactive protein [CRP]) and clinical assessments (6-minute walking test, the New York Heart Association [NYHA] class and the Minnesota Living with Heart Failure Questionnaire [MLHFQ]). MR and immunological analyses were performed in central and blinded core-laboratories (University Hospitals Leuven, Belgium and Ramón y Cajal University Hospital, Madrid, Spain, respectively). Patients attended hospital visits at 1 week and at 1, 3, 6 and 12 months. Telephone visits were scheduled after 2, 4, 5 and 9 months.

Statistical analysis.
Statistical analyses and analysis sets were fully defined prior to treatment allocation un-blinding in a Statistical Analysis Plan. Since this was an early-stage safety exploratory study, no statistical sample size calculation was performed on a priori superiority or non-inferiority hypothesis in terms of efficacy. With 34 patients randomized to the treatment group and an expected MACE rate of 25%, there was an 88% chance that the exact binomial confidence interval for the event rate would have a half-width of 0.166 or less. In addition, the study power was a priori tested to ensure sensitivity for detecting differences in infarct size of the magnitude previously reported in the CADUCEUS trial.[11]

Statistical analyses were conducted on patients in the double-blind randomized phase of the trial, including the last two patients from the dose-escalation phase who received $35x10^6$ CSC. A safety analysis set (SAS) was defined that included all patients who received the target dose (either in the randomized or in the dose-escalation phase) or placebo, for the assessment of all safety outcomes and adverse events. A full analysis set (FAS) was defined based on the intention-to-treat principle, including all randomized patients except for those who did not receive the study medication and did not have any post-baseline data. A per-protocol set (PPS) included all patients in the FAS who did not have any major protocol violations. Efficacy data were analysed for both the FAS and the PPS. Since results for the PPS did not differ significantly from those for the FAS, no results will be presented for the PPS.

Given that there were no missing data for the primary (safety) endpoints, event rates were estimated using observed percentages with exact 95% confidence intervals (CI). The treatment effect was estimated by the difference in event-rates between the two groups at 6 and 12 months with the exact 95% CI. Comparisons of the two treatment groups were done using a Fisher's exact test. Secondary safety endpoints were analysed using Kaplan-Meier methodology. The 95% CI and p-values were calculated using the z-statistic.

The secondary efficacy data were analysed with a constrained Longitudinal Data Analysis model using generalized estimating equations (GEE).[12] The correlations between visits were modelled using an unstructured variance-covariance matrix. Even though some data points were missing (mostly due to bad MR image quality or reluctance of patients to go into the MR scanner), GEE provide unbiased estimates of the treatment effect in the presence of missing data, if the assumption of "Missingness at Random" holds. For the main efficacy endpoint of interest, infarct size (as percentage of LV mass) at 12 months, the following a priori defined exploratory subgroup analyses were performed: age, LVEF, infarct size, infarct-related artery, presence of microvascular obstruction and myocardial haemorrhage by MR and donor identity.

Since the aim of this study was to demonstrate feasibility and safety of the study treatment, there were no a priori defined hypotheses regarding the efficacy of the treatment of interest. Hence, the focus of the efficacy analyses was on the estimation of results within treatment groups and of differences between groups and the associated 95% CI. Any significance testing was done purely for illustrative purposes.
Although this study had two primary endpoints, an increase in the Type I error above 5% due to multiple testing was not considered an issue since this was a safety study: increasing the chance of detecting a safety issue by not adjusting the significance level (ie, to 2.5%) can be considered a conservative approach. Therefore, no adjustment for multiple testing was made and the two primary endpoints were assessed at a significance level of 5%. For the secondary efficacy endpoints, due to the exploratory nature of the efficacy analyses, no adjustment for multiple comparisons were made. All analyses were performed using SAS software (version 9.41).

RESULTS

Bioequivalence between batches.

The phenotypic characterization of AlloCSC-01 was carried out by flow cytometry, quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA) (Table 1). AlloCSC-01 were isolated from three different donors (one 66-year-old male and two females, aged 44 and 62 respectively). These three batches showed comparable (positive or negative) levels of expression of cardiac progenitor, mesenchymal, endothelial and hematopoietic surface markers. AlloCSC-01 were positive for CD90, CD105, CD146, CD166 and SSEA-1 while lacking the expression of CD11b, CD34 and CD45. In addition, the expression of GATA4, SOX17, FLK1, CD31, NESTIN and TBX5 was confirmed by qPCR. The expression of GATA4 and SOX17, involved in heart development,[13, 14] distinguishes CSC from mesenchymal stem cells (MSC) and diploid fibroblasts. CSC secretion of soluble factors involved in immunoregulation, cardiac regeneration, stem cell activation, angiogenesis or cell differentiation was studied by ELISA. The three batches of AlloCSC-01 released comparable levels of CCL2, HGF, VEGF, TGF-β1 and IGF-1. The capacity of AlloCSC-01 to be activated by an inflammatory environment and to modulate the function of the immune cells was characterized in vitro (Figure 1).

Conditioned supernatants from the three AlloCSC-01 batches equally promoted the migration of monocytes in vitro in a transwell system. The cells were activated by exposure to IFNγ, which resulted in upregulation of HLA-I and PD-L1 and in induction of IDO and HLA-II. The expression of PD-L1 and IDO, with important immunomodulatory properties, could be relevant for the immunoregulatory effects of AlloCSC-01 in the context of STEMI. Finally, the three batches of AlloCSC-01 demonstrated a similar capacity to inhibit lymphocyte proliferation in vitro. See Online Supplement for a more detailed description of HLA donor typing, surface markers, c-kit status and comparative genomic hybridization results.

Table 1. AlloCSC01 phenotypic characterization and bioequivalence of the three batches. Isolated from three different donors, the three batches of AlloCSC01 showed similar levels of expression of mesenchymal, endothelial and hematopoietic surface markers by flow cytometry. Several genes were analyzed by qPCR, using BM-MSC as reference control cell line. Secretome was analyzed by the ELISA technique in the AlloCSC-01 conditioned medium. Non-significant differences were observed between batches in terms of bioequivalence.

	Marker	Batch 1	Batch 2	Batch 3
Analysis by flow cytometry (% of positive cells)	CD90	98.0	97.9	77.1
	CD105	99.8	99.7	99.7
	CD166	99.6	97.7	98.8
	CD146	48.9	60.0	39.1
	SSEA-1	26.0	20.1	34.9
	FSP	6.6	7.0	3.5
	CD144	5.6	1.2	8.6
	CD34	0.2	2.7	1.2
	CD11b	<0.1	0.3	0.3
	CD45	<0.1	0.6	1.3
Analysis by qPCR fold change (log_2 RQ), relative to BM-MSC values	GATA4	7.5	6.5	5.5
	SOX17	8.6	8.3	7.9
	FLK1	4.8	5.5	4.9
	CD31	1.7	1.2	3.0
	NESTIN	4.8	4.1	4.2
	TBX5	3.8	1.2	1.5
Secretome by ELISA (ng/mL)	CCL2	81.4	41.6	45.7
	HGF	25.9	9.4	3.3
	VEGF	4.45	1.43	2.31
	TGF-β1	1.64	1.16	2.31
	IGF-1	0.03	0.03	0.06

AlloCSC-01: allogeneic cardiac stem cells; qPCR: quantitative polymerase chain reaction; BM-MSC: bone marrow-derived mesenchymal cells; ELISA: enzyme-linked immunosorbent assay; CD: cluster of differentiation; SSEA-1 (CD15): stage-specific embryonic antigen-1; FSP: fibroblast surface marker; CCL2 (MCP1): chemokine receptor 2 (monocyte chemoattractant protein 1); HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor; TGF-β1: transforming growth factor β1; IGF-1: insulin-like growth factor-1.

Patients.

From June 2014 to November 2016, a total of 66 patients provided written informed consent and were screened for the double-blind phase in seven centers in Spain and one center in Belgium (Figure 2). Finally, 49 patients were randomized to receive either AlloCSC-01 (33 patients) or placebo (16 patients). Two patients allocated to the AlloCSC-01 group did not receive any treatment because of one coronary stenosis in the infarct-related artery that was considered an indication for revascularization prior to cell infusion and one consent withdrawal. Thus, the SAS consisted of 49 patients: 47 randomized patients who received the assigned therapy plus the two non-randomized patients from the dose escalation phase who received the highest dose. The 47 randomized patients comprised the FAS population and all of them completed the 12-month follow-up visit. Three patients in the AlloCSC-01 group and no patient from the placebo group had major protocol violations, resulting in the PPS of 44 patients (Figure 2).

Baseline and index PCI characteristics were well balanced between groups (Table 2). All female patients were allocated to the AlloCSC-01 group. Of note, our population included young patients with few risk factors (mostly smokers and with a low prevalence of diabetes), predominantly with single-vessel disease and who underwent successful revascularization within 4 hours of symptom onset (Table 2).

Table 2. Baseline characteristics (safety analysis set). Data expressed as mean ± standard deviation except otherwise indicated. p=NS for all between-group comparisons.

	AlloCSC-01 n=33	**Placebo n=16**
Demographics and medical history		
Age, years	56±12	55±8
Age < 55, n (%)	14 (45)	9 (56)
Female sex, n (%)	4 (12)	0
Any cardiovascular risk factor, n (%)	27 (82)	16 (100)
Familiar history of premature CAD, n (%)	4 (12)	3 (19)
Hypertension, n (%)	14 (42)	6 (38)
Hypercholesterolemia, n (%)	13 (39)	5 (31)
Insulin dependent diabetes mellitus, n (%)	0	1 (6)
Non-insulin dependent diabetes mellitus, n (%)	2 (6)	2 (13)
Current smoker, n (%)	18 (55)	8 (50)
Prior smoker, n (%)	5 (15)	3 (19)
Prior NSTEMI, n (%)	1 (3)	0
Prior PCI, n (%)	1 (3)	0
Prior CABG, n (%)	0	0
Peripheral artery disease, n (%)	0	0
Stroke, n (%)	2 (6)	0
Renal failure, n (%)	1 (3)	1 (6)
Physical examination		
Weight, kg	81±16	84±12
Heart rate, beats/min	76±18	81±15
Systolic blood pressure, mmHg	131±22	134±22
Diastolic blood pressure, mmHg	78±15	87±18
Killip class, n (%)		
I	28 (85)	14 (88)
II	5 (15)	2 (13)
III or IV	0	0
Index PCI procedure		
Type of reperfusion strategy		
Primary PCI, n (%)	28 (85)	13 (81)
Post-fibrinolysis PCI, n (%)	5 (15)	3 (19)
Infarct-related coronary artery		
LAD, n (%)	29 (88)	14 (88)
Non-LAD, n (%)	4 (12)	2 (13)
TIMI flow grade on angiography before PCI		
0, n (%)	28 (85)	12 (75)
1, n (%)	3 (9)	2 (13)
2, n (%)	1 (3)	2 (13)
3, n (%)	1 (3)	0

Time from symptom onset to flow restoration min, median (IQR)	216 (150-285)	221 (134-430)
Type of stent in infarct-related coronary artery		
Bare metal stent, n (%)	2 (6)	1 (6)
Drug-eluting stent, n (%)	31 (94)	15 (94)
Stent diameter, mm	3.3±0.4	3.4±0.3
Stent length, mm	22.0±8.1	20.7±9.2
Additional revascularization of non-infarct related coronary arteries before cell infusion, n (%)	11 (33)	6 (38)

AlloCSC-01: allogeneic cardiac stem cells; CABG: coronary artery bypass grafting; CAD: coronary artery disease; IQR: interquartile range; LAD: left anterior descending; PCI: percutaneous coronary intervention; NSTEMI: non-ST-segment elevation myocardial infarction; TIMI: Thrombolysis In Myocardial Infarction.

Primary safety endpoints.

All 49 patients in the SAS received the full intended treatment of $35x10^6$ CSC or placebo, reconstituted in 18 mL. No signs of ischemia, anaphylaxis, hemodynamic instability or ventricular arrhythmias were observed. One patient in the placebo group showed transitory TIMI flow grade 2 after infusion, but rescue medication was not required. The mean duration of the infusion was comparable between the AlloCSC-01 and placebo groups (19.0 ± 3.9 min versus 19.6 ± 4.8 min, respectively). Importantly, no AE, deaths or MACE (all-cause death, reinfarction, hospitalization due to HF, sustained ventricular tachycardia, ventricular fibrillation and stroke) were registered within 30 days after treatment administration in either group.

Secondary safety endpoints.

There were no deaths or MACE observed within 6 and 12 months after study treatment administration in any of the treatment groups.

Twenty-two patients (67%) in the AlloCSC-01 group and 9 patients (56%) in the placebo group suffered an AE during the first year (Table 2). One patient in the placebo group was diagnosed with basal cell carcinoma, which required surgical resection. Among all AE, 5 in the AlloCSC-01 group and 2 in the placebo group were considered to be possibly or probably related to study treatment, and all occurred during the first month. In the AlloCSC-01 group, 3 patients suffered pyrexia, 1 patient developed a catheter-related site hematoma and 1 patient a transient allergic dermatitis. In the placebo group, 2 patients developed a transient rash. The 3 cases of pyrexia were classified as mild to moderate in intensity and self-limiting, recovering within a few hours. These 3 patients had received cells from the same cell batch and donor, and anti-HLA antibodies were not detected in any of them.

During the study, 8 patients (24%) in the AlloCSC-01 group and 2 patients (13%) in the placebo group had serious AE (SAE). Only 2 SAE were considered to be possibly related to study treatment (one allergic dermatitis in the AlloCSC-01 group and one episode of rash in the placebo group). Both occurred within the first month and resolved spontaneously with no additional measures. In the AlloCSC-01 group, 3 patients suffered non-cardiac chest pain and 1 patient in each group suffered lower gastrointestinal hemorrhage, allergic dermatitis (not related to study treatment), abdominal pain, acute cholecystitis and nephrolithiasis. In the placebo group, 1 patient experienced palpitations and 1 patient had rash and cellulitis.

There were no fatal AE during the course of the study and no patients were withdrawn from the study because of AE. Creatine kinase (CK) levels decreased progressively after the infusion of AlloCSC-01 (from 227±175 IU/L pre-treatment to 175±100 IU/L at 8 hours to 151±62.7 IU/L at 24 hours) as well as after the infusion of placebo (from 153±44.4 IU/L pre-treatment to 125±41.2 IU/L at 8 hours to 117±30.7 IU/L at 24 hours). A stable trend was observed for CK-MB in both groups (from 2.8±1.5 μg/L pre-treatment to 2.4±1.3 μg/L at 8 hours to 2.8±1.8 μg/L at 24 hours in the AlloCSC-01 group; from 3.2±0.8 μg/L pre-

treatment to 2.5±0.9 μg/L at 8 hours to 2.7±0.9 μg/L at 24 hours in the placebo group). A decreasing trend was also observed for troponin T (from 2.1±1.2 μg/L pre-treatment to 1.7±1.2 μg/L at 8 hours to 1.3±1.0 μg/L at 24 hours in the AlloCSC-01 group and from 2.5±1.6 μg/L pre-treatment to 1.8±1.3 μg/L at 8 hours to 1.6±1.0 μg/L at 24 hours in the placebo group). Electrocardiographic (ECG) exams were representative of the patient population and raised no safety signals of recurrent ischemia during the infusion procedure and until discharge.

A systematic immunological sub-study was conducted to detect anti-HLA antibodies at several time points after intracoronary infusion of AlloCSC-01. Seven patients (14%) had preexisting anti-HLA antibodies at baseline (5 in the AlloCSC-01 arm and 2 in the placebo arm). Those pre-sensitized patients who received AlloCSC-01 exhibited a negative virtual crossmatch against CSC by the Labscreen single antigen assay and did not boost the pre-existing humoral response. After AlloCSC-01 administration, 2 patients (7%) developed "*de novo"* donor-specific antibody (DSA) responses, as confirmed by single antigen technology (>20,000 standard fluorescence intensity). Peak DSA levels were reached at 1 month in one patient and at 3 and 6 months in the other patient, and were cleared by 12 months. None of these patients with preexisting antibodies or who developed "*de novo*" antibodies experienced any hypersensitivity-related AE nor any other specific AE that could be potentially attributable to an immunogenic response.

Secondary efficacy endpoints.

Mean infarct size as a percentage of the LV mass decreased over time in both treatment groups (Table 3, Figure 3). Estimated absolute change in infarct size from baseline to 1-year was -15.6% (95% IC -18.3% to -12.8%) in the AlloCSC-01 group and -13.3% (-16.7% to -9.8%) in the placebo group. Thus, the estimated treatment effect of AlloCSC-01 on absolute changes in infarct size was -2.3% (-6.5% to 1.9%) in the FAS and -1.6% (-5.8% to 2.6%) in the PPS. The largest reductions in infarct size occurred within the first month after treatment irrespective of treatment allocation, and there were no significant differences in infarct size between the two treatment groups at 1 or 6 months either.

Table 3. Main magnetic resonance imaging secondary-endpoint efficacy results at baseline, 1, 6 and 12 months in the full analysis set. Data expressed as mean ± standard deviation; estimated changes are expressed as estimated averages with 95% CIs and were obtained using a constrained longitudinal data analysis model using general estimating equations. p=NS for all comparisons.

	AlloCSC-01 n=31	**Placebo n=16**
Infarct size (%)		
Baseline (n)	38.9±9.2 (31)	38.5±9.7 (16)
1 month (n)	29.2±11.5 (30)	30.8±11.4 (14)
6 months (n)	26.8±12.0 (29)	26.9±10.1 (14)
12 months (n)	22.7±9.9 (30)	25.4±9.3 (15)
Estimated absolute change from baseline to 1 month (95% CI)	-9.9 (-12.9 to -6.9)	-9.8 (-14.4 to -5.2)
Estimated absolute change from baseline to 6 months (95% CI)	-12.2 (-15.4 to -9.1)	-10.4 (-14.6 to -6.1)
Estimated absolute change from baseline to 12 months (95% CI)	-15.6 (-18.3 to -12.8)	-13.3 (-16.7 to -9.8)
Left ventricular ejection fraction (%)		
Baseline (n)	37.5±5.1 (31)	36.6±3.4 (16)
12 months (n)	44.7±8.7 (31)	45.6±5.8 (16)
Estimated absolute change from baseline to 12 months (95% CI)	7.7 (5.1 to 10.2)	8.6 (6.2 to 11.1)
LV end-systolic volume index (mL/m^2)		
Baseline (n)	56.9±13.0 (31)	57.7±8.3 (16)
12 months (n)	56.8±23.0 (31)	54.8±11.9 (16)
Estimated absolute change from baseline to 12 months (95% CI)	0.9 (-5.2 to 7.1)	-3.5 (-9.4 to 2.5)
LV end-diastolic volume index (mL/m^2)		
Baseline (n)	90.2±15.6 (31)	91.1±11.1 (16)
12 months (n)	100.4±26.3 (31)	100.3±14.5 (16)
Estimated absolute change from baseline to 12 months (95% CI)	10.1 (3.8 to 16.4)	9.3 (3.0 to 15.6)
Wall motion score index		
Baseline (n)	3.2±0.5 (31)	3.4±0.3 (16)
12 months (n)	2.7±0.8 (31)	2.6±0.6 (16)
Estimated absolute change from baseline to 12 months (95% CI)	-0.6 (-0.9 to -0.4)	-0.7 (-1.1 to -0.5)

AlloCSC-01: allogeneic cardiac stem cells; CI: confidence interval; LV: left ventricular.

No significant differences between groups were observed in terms of ventricular volumes, LVEF or regional wall motion at 1 year (Table 3, Figure 3). There was a similar increase from baseline in LV end-diastolic volume index (10 mL/m^2 versus 9 mL/m^2) and LVEF (7.7% versus 8.6%) in AlloCSC-01 versus placebo, respectively. LV end-systolic volume index only changed minimally from baseline (0.9 mL/m^2 versus -3.5 mL/m^2) in AlloCSC-01 versus placebo, respectively. Microvascular obstruction was equally present in 17 patients in the AlloCSC-01 group (60% of available data) and in 11 patients in the placebo group (73% of available data). Pre-specified analyses were performed to assess potential interactions between the treatment effect on the change in infarct size and a number of binary covariables (Figure 4). However, no differences were found in the binary interaction analyses for age ≤ 55 years (median value),

LVEF ≤ 37% (median value), infarct size ≤ 39% (median value) or donor source. The other pre-specified subgroups were not included in the analysis due to the low number of events (<5).

Pretreatment levels of CRP were similar in both groups (16.3 [11.6 to 31.5] mg/L versus 14.1 [9.5 to 21.8] mg/L) in AlloCSC-01 versus placebo, respectively. However, at day 7 median CRP levels were lower in the AlloCSC-01 group (5.4 [3.0 to 15.0] mg/L) than in the placebo group (8.1 [3.3 to 35.5] mg/L) (p=0.04). At 1 month, CRP levels had further decreased in both AlloCSC-01 (2.0 [1.0 to 3.7] mg/L) and placebo (2.5 [1.0 to 4.0] mg/L) groups (p=0.097 for the difference between treatment groups). Pretreatment levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) were also similar between AlloCSC-01 (1101.0 [518.9 to 1413.0] ng/L) and placebo (737.0 [481.0 to 1618.0] ng/L) groups. There were no significant differences in NT-proBNP levels between groups in the change from baseline to 12-month follow-up (-72.9 [-83 to -57.8] mg/L in AlloCSC-01 versus -74.1 [-82.9 to -52.4] mg/L in placebo, p=0.9).

Both AlloCSC-01 and placebo-treated patients improved functional class with a similar rise in 6-minute walking test results at 12 months (from 438±128 to 555±181 meters in the AlloCSC-01 group and from 407±99 to 524±100 meters in the placebo group, p=0.6). No significant changes were observed in the MLHFQ score in any group at 12 months. Similarly, the proportion of patients with NYHA Class I and II remained comparable over time in both groups at 3, 6 and 12 months, with no significant differences at any time point.

DISCUSSION

In this study, we show that allogeneic cardiac stem cells isolated from human heart biopsies, cultured and processed as AlloCSC-01 are safe and well tolerated when administered through the intracoronary route in the infarct-related artery of patients with STEMI and at risk of developing heart failure. However, with respect to exploratory efficacy surrogate endpoints, we did not observe a CSC-mediated incremental reduction in infarct size or improvement in LV remodeling at 1-year follow-up.

In search of better cell types for myocardial repair after STEMI, autologous CSC and cardiosphere-derived cells (CDC) have been investigated in small-scale pilot clinical trials with encouraging safety results.[11, 15] While efficacy data necessitate larger scale clinical outcome trials, an autologous approach imposes significant constraints because of the expense and inflexibility related to patient-specific tissue harvesting, cell expansion and quality control. Moreover, progenitor cell efficacy and biological regenerative potential may vary with co-morbidities and the presence of multiple risk factors.[16, 17] The above limitations have paved the way to explore allogeneic treatment paradigms ("off the shelf" cell products). Our study was the first to administer allogeneic CSC (AlloCSC-01) of comparable biological activity early (5-7 days) after STEMI in carefully selected patients at increased risk, and therefore a thorough assessment of safety was the main objective of the trial. As a pre-specified first step in our clinical development plan, we first monitored the safety of 3 different doses of AlloCSC-01 during a dose-escalation phase. We found no safety concerns at any dose level, and proceeded with the randomized clinical trial using a preclinically defined target dose of $35x10^6$ CSC.[10] This pilot study met its primary objective by demonstrating that AlloCSC-01 can be safely administered in STEMI patients. The intracoronary infusion of AlloCSC-01 was successful and showed no immediate clinical, hemodynamic or arrhythmic safety issues, when compared to placebo. Cardiac enzymes and ECG monitoring further demonstrated that neither the procedure nor the cellular product caused any additional damage to the myocardium. Careful clinical follow-up in all patients revealed no deaths or MACE within 30 days of treatment administration, and these reassuring results extended up to 12 months of follow-up. The low event rate in placebo patients may be explained by the good short and mid-term prognosis of our population, which was –though unselected– relatively young and

previously healthy with limited co-morbidities, showing better outcomes than reported in larger published series.[18]

Some SAE were observed during study conduct, but only 2 were considered possibly related to the study treatment. Both occurred within the first month, were brief and required no specific treatment. Importantly, the patient with a SAE after cell infusion (dermatitis) was not presensitized to AlloCSC-01 and developed no DSA after their administration. Given the allogeneic approach of the CAREMI trial, it is of particular relevance that no patients experienced immune-related AE in the follow-up. Indeed, CSC induced anti-AlloCSC-01 antibody responses only in 2 patients, specifically against the HLA II and against the C locus of the HLA I complex. In these 2 cases the humoral response was not sufficient to result in a durable and clinically relevant concentration of DSA (no hypersensitivity-related events observed). Of interest, exploratory analysis of inflammatory markers showed a significantly greater reduction in CRP levels 1 week and 1 month after CSC transfer. Unfortunately, the low number of patients in our study does not allow to draw definitive conclusions about the relationship between the immune response and the outcomes of the trial, an issue that warrants larger follow-up studies in light of the recently established benefit of interleukin-1β monoclonal antibodies in patients with acute coronary syndromes.[19] Finally, the low immunogenicity of allogeneic stem cells is in agreement with immunological analyses in the POSEIDON-DMC trial of allogeneic mesenchymal stem cells in dilated cardiomyopathy patients and will undoubtedly facilitate future larger-scale trials.[20]

Designed as a phase I/II safety and feasibility clinical trial, CAREMI was underpowered to demonstrate differences in efficacy as specified using MR-based surrogate endpoints. Infarct size was selected as the main exploratory secondary efficacy endpoint because of its strong association with all-cause mortality and hospitalization for HF.[8] We found that treatment with AlloCSC-01 had a small effect on the change in infarct size, as well as on LV volumes and LVEF. MR is widely accepted as the gold standard for cardiac imaging in the setting of STEMI and LV dysfunction and has been extensively used to assess surrogate efficacy endpoints in cell therapy trials, allowing for smaller sample sizes and shorter follow-up times.[21] However, our efficacy results are in agreement with those of other negative stem cell trials in STEMI,[22-25] and meta-analyses in this setting have recently shown variable effects of several cell types on LVEF, volumes or infarct size,[26] when measured by different imaging modalities including MR.[27-30] A possible explanation for the lack of additional benefit of AlloCSC-01 on MR-based surrogate endpoints may relate to the dose or timing of administration. We used an allogeneic source of CSC and infused cells in the acute phase of STEMI, in contrast with previous trials where autologous CSC were administered 113±4 days after coronary artery bypass grafting (CABG) in chronic myocardial infarction patients,[15] or autologous CDC were infused at 1.5-3 months after STEMI.[11] We decided to use this time-window (5-7 days) because of high mortality rates in swine models when injecting the cells at "zero time" right after reperfusion and because of good retention rates of ^{18}F-FDG-labelled CSC (18%) at 4 hours with PET when injecting the cells at 5-7 days (data not shown). Alternatively, the dose of administered AlloCSC-01 may not suffice to counteract the molecular, cellular and tissue changes that take place in the myocardium during the first weeks after STEMI and that activate the complex remodeling process. However, the maximum administered dose was that allowed by the competent authorities and EMA. The excellent results of current aggressive prompt reperfusion strategies and standard-of-care pharmacotherapy in STEMI patients markedly reduced the window of opportunity for any novel therapeutic intervention.[31] Importantly and consistent with successful contemporary STEMI care, we observed a large reduction in infarct size in our control population and the virtual absence of adverse LV remodeling at 1 year. Recent studies have defined clinically relevant adverse LV remodeling as an increase of more than 20% in LV end-diastolic volume,[32] which is more than twice the increase observed in our control patients. Therefore, in order to demonstrate any positive efficacy result, follow-up studies should focus on patients with better identified risk of adverse remodeling and investigate higher doses of CSC, new strategies including repetitive administration of CSC,[33] combination of cells[34] or potentially more efficient intramyocardial or intrapericardial delivery routes.[35]

Study limitations.

The small sample size and the design of the trial allowed only valid conclusions on safety but no definitive evaluation of efficacy. The 1-year follow-up may not have been long enough to fully appreciate clinical events or the dose of infused cells not sufficiently high to detect potential benefit on surrogate MR-based parameters. The timeframe for AlloCSC-01 infusion was derived from previous experiences,[10] but no specific studies were performed in humans to better define the optimal timing for the administration of this type of CSC in the setting of acute STEMI.

Conclusion.

The CAREMI trial contributes to the growing body of clinical evidence supporting the feasibility and the safety of intracoronary CSC infusion in the acute phase (5-7 days) of STEMI. No clinically significant cardiac or immunological events following allogeneic cell infusion were observed throughout follow-up. Although not powered to show differences in efficacy endpoints, our study demonstrated no incremental benefit in infarct size reduction, indices of LV remodeling, laboratory assessments, functional class or quality of life scores between CSC and placebo-treated patients. Adequately powered studies with larger populations at increased risk for adverse remodeling would be needed if we ever aim at demonstrating the potential efficacy of allogeneic CSC on structural parameters and clinical outcome in STEMI.

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DISCLOSURES
IG, MM, IP, ML, EL, JLA, OD and LC are employed by Coretherapix S.L.U. DC, SJ and FFA are members of the Tigenix Scientific Advisory Board. Coretherapix S.L.U. is part of the Tigenix Group since July 2015.

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FIGURE LEGENDS

Figure 1. Functional bioequivalence analyses of AlloCSC01 in the three batches. A) Migration of monocytes in response to CSC conditioned media (results presented as mean ± standard deviation). B) Expression of immunomodulatory molecules (IDO, PD-L1, HLA-I and HLA-II) in AlloCSC01 at baseline and after 24h of IFNγ stimulation. C) Immunoregulatory capacity of AlloCSC-01, as assessed by the inhibition of lymphocyte proliferation from PBMC. For further methods description see Online Supplement. AlloCSC-01: allogeneic cardiac stem cells; IFNγ: interferon γ; IDO: indoleamine 2,3-dioxygenase; PD-L1: programmed death-ligand 1 (CD274); HLA-I: type I human leukocyte antigen; HLA-II: type II human leukocyte antigen; PBMC: peripheral blood mononuclear cells; DI: division index; % IP: percentage of inhibition of proliferation.

Figure 2. Trial profile. Diagram showing the number of patients screened, excluded, randomized and evaluated for clinical follow-up. AlloCSC-01: allogeneic cardiac stem cells; IS: infarct size; LVEF: left ventricular ejection fraction; PCI: percutaneous coronary intervention; SAS: safety analysis set; FAS: full analysis set; PPS: per protocol set.

Figure 3. Evolution of infarct size, left ventricular ejection fraction, left ventricular end-systolic volume index and left ventricular end-diastolic volume index by magnetic resonance at baseline, 1, 6 and 12 months. The results represent the estimated averages with 95% confidence intervals and were obtained using a constrained longitudinal data analysis model using general estimating equations. LV: left ventricular; LVEF: left ventricular ejection fraction; LVESVi: left ventricular end-systolic volume index; LVEDVi: left ventricular end-diastolic volume index; B/L: baseline; M1: month 1; M6: month 6; M12: month 12.

Figure 4. Subgroup analysis. The values shown correspond to the changes of infarct size from baseline to 1-year follow-up among patients undergoing AlloCSC-01 or placebo infusion in the pre-specified subgroups. Median values were used as cut-off points for age, left ventricular ejection fraction and infarct size. Subgroups with insufficient number of patients in any category have not been included in the forest plot. CSC: cardiac stem cells; EF: ejection fraction.

NOVELTY AND SIGNIFICANCE

What Is Known?

- Although safe, the regenerative efficacy of first-generation stem cells for ST-segment elevation myocardial infarction (STEMI) has been shown inconsistent.
- Exogenous cardiac stem cells (CSC) exert positive effects on myocardial protection and repair with an immunomodulatory behavior in preclinical models.
- Allogeneic sources can be produced in large quantities beforehand and may be administered "off-the-shelf" early during the acute phase of myocardial ischemia.

What New Information Does This Article Contribute?

- This article reports one of the first-in-man experiences with early administration of allogeneic CSC in patients with STEMI and at risk of adverse remodeling.
- The intracoronary infusion of these cells 5 to 7 days after STEMI is safe from a clinical, hemodynamic and immunological standpoint (primary endpoint).
- Allogeneic CSC, at one single dose of $35x10^6$, did not produce benefits in terms of ventricular remodeling and healing (secondary exploratory endpoint).

After inconclusive results with old types of autologous stem cells to achieve myocardial regeneration and repair, allogeneic CSC have been postulated to improve the outcomes of patients with STEMI because of their cardiac protective, immunoregulatory and regenerative properties. The main results of this phase I/II clinical trial with early administration of allogeneic CSC through the intracoronary route demonstrate the safety of this approach after comprehensive clinical and immunological surveillance. Furthermore, and despite being exploratory, interesting neutral results on magnetic resonance-based efficacy parameters are presented. The trial provides new mechanistic insights into the biology of allogeneic CSC-based therapeutic products and entails two significant implications. First, it proves that this type of allogeneic cells can be safely administered in humans. Second, it suggests that allogeneic CSC, at doses of $35x10^6$ cells and with one single intracoronary injection, do not modify the healing process of the left ventricle after STEMI (ie, infarct size) and do not counteract adverse remodeling (ie, left ventricular volumes and ejection fraction) in the current reperfusion era. These results may also have important implications for translational research with preclinical models, eventually aiding the design of future clinical trials.

FIGURE 1

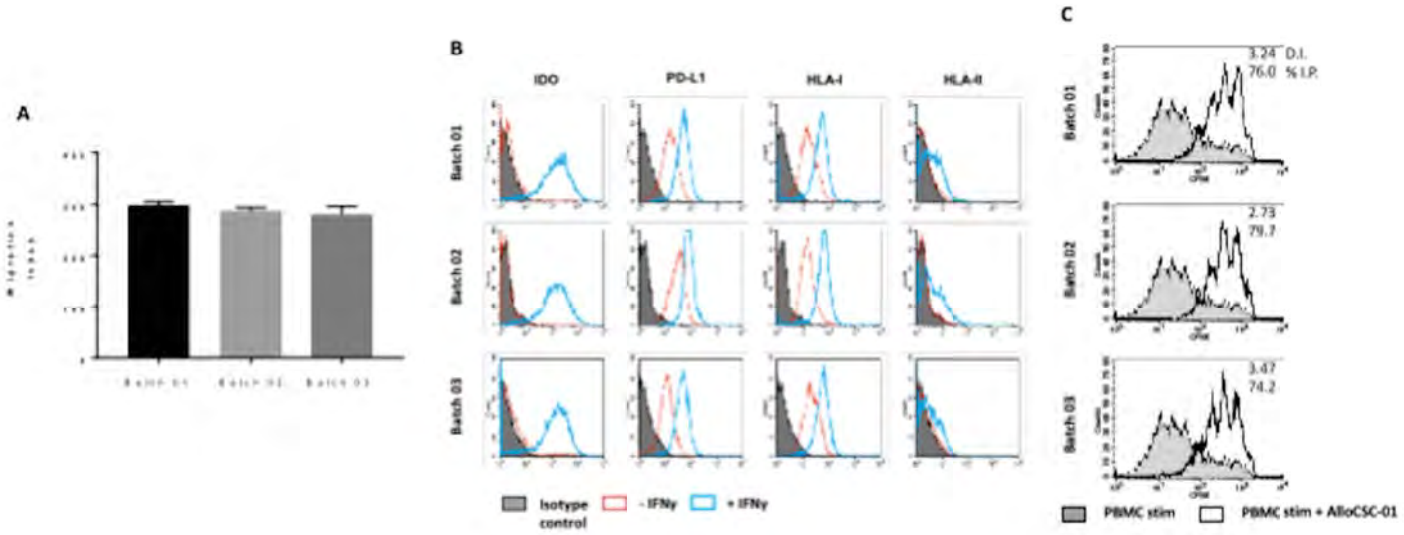
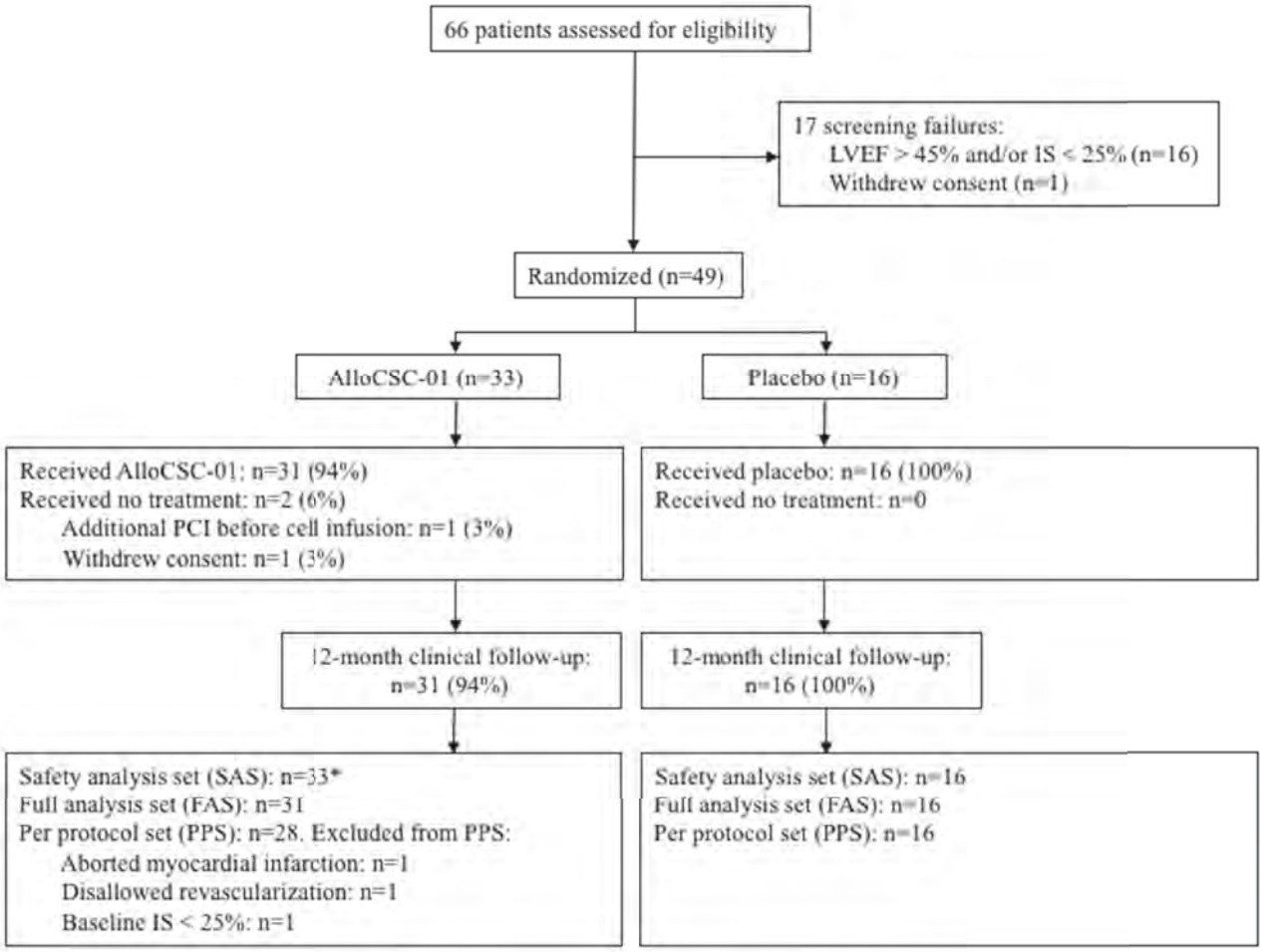
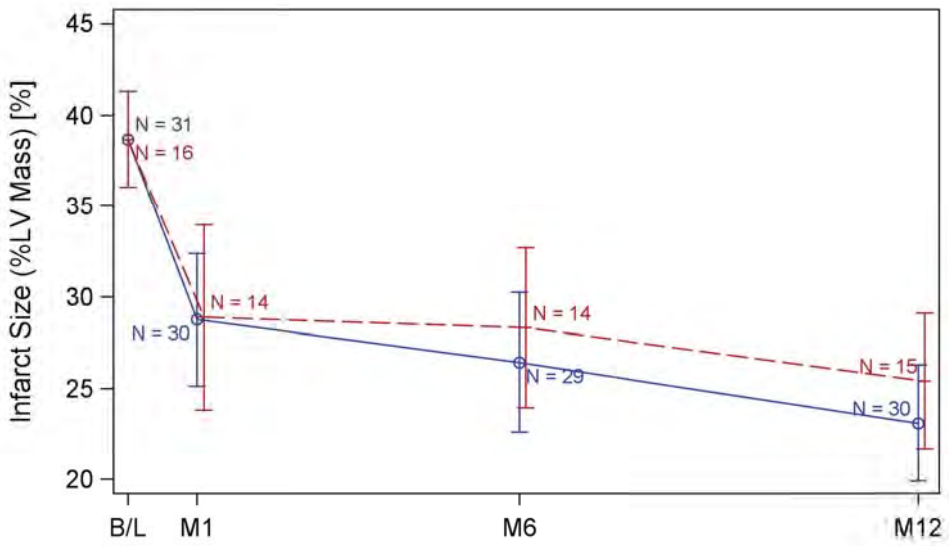


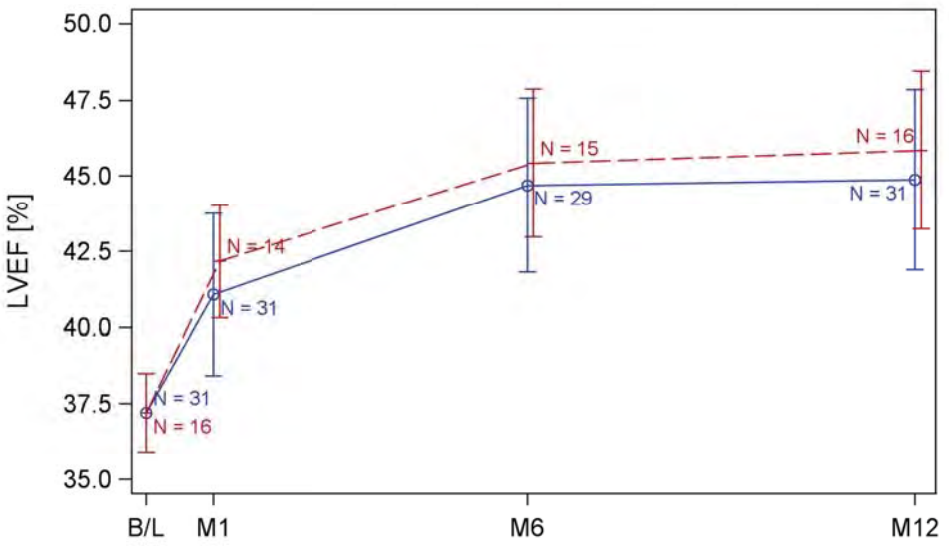
FIGURE 2

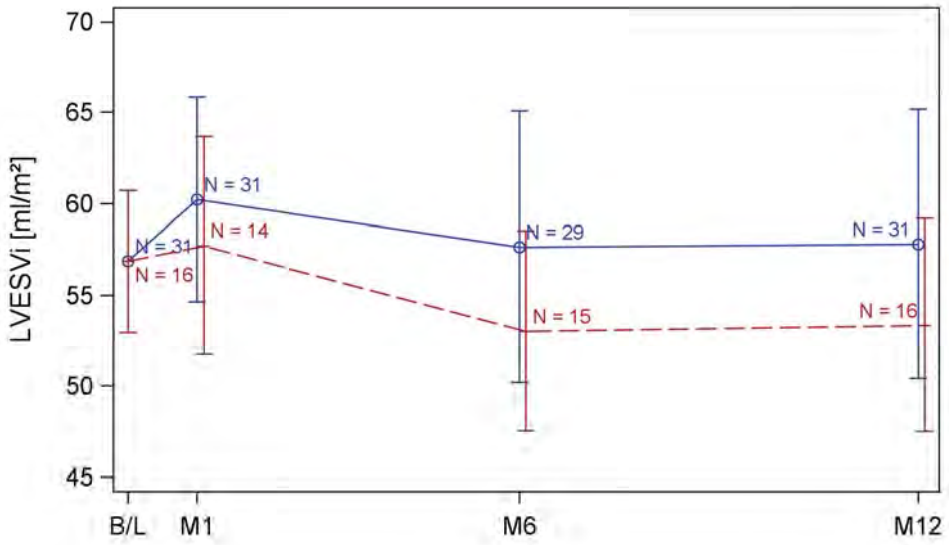


*Includes the last two patients from the dose-escalation phase who received $35x10^6$ AlloCSC-01

FIGURE 3







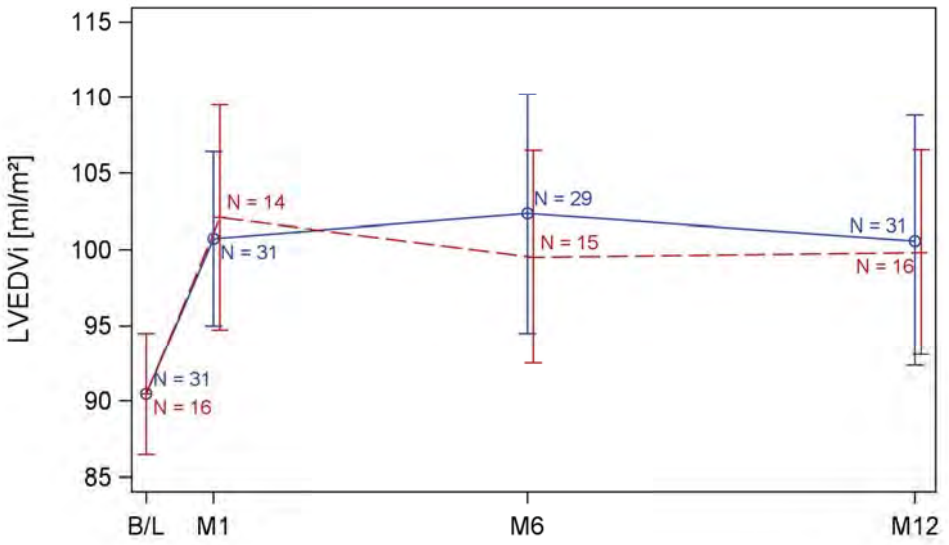


FIGURE 4