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Cardiopoietic cell therapy for advanced ischemic heart failure: results at 39 weeks of the prospective, randomized, double blind, sham-controlled CHART-1 clinical trial

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Aims Cardiopoietic cells, produced through cardiogenic conditioning of patients' mesenchymal stem cells, have shown preliminary efficacy. The Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial aimed to validate cardiopoiesis-based biotherapy in a larger heart failure cohort.

Methods and results This multinational, randomized, double-blind, sham-controlled study was conducted in 39 hospitals. Patients with symptomatic ischemic heart failure on guideline-directed therapy ($n = 484$) were screened; $n = 348$ underwent bone marrow harvest and mesenchymal stem cell expansion. Those achieving > 24 million mesenchymal stem cells ($n = 315$) were randomized to cardiopoietic cells delivered endomyocardially with a retention-enhanced catheter ($n = 157$) or sham procedure ($n = 158$). Procedures were performed as randomized in 271 patients ($n = 120$ cardiopoietic cells, $n = 151$ sham). The primary efficacy endpoint was a Finkelstein–Schoenfeld hierarchical composite (all-cause mortality, worsening heart failure, Minnesota Living with Heart Failure Questionnaire score, 6-min walk distance, left ventricular end-systolic volume, and ejection fraction) at 39 weeks. The primary outcome was neutral (Mann–Whitney estimator 0.54, 95% confidence interval [CI] 0.47–0.61 [value > 0.5 favours cell treatment], $P = 0.27$). Exploratory analyses suggested a benefit of cell treatment on the primary composite in patients with baseline left ventricular end-diastolic volume 200–370 mL (60% of patients) (Mann–Whitney estimator 0.61, 95% CI 0.52–0.70, $P = 0.015$). No difference was observed in serious adverse events. One (0.9%) cardiopoietic cell patient and 9 (5.4%) sham patients experienced aborted or sudden cardiac death.

Conclusion The primary endpoint was neutral, with safety demonstrated across the cohort. Further evaluation of cardiopoietic cell therapy in patients with elevated end-diastolic volume is warranted.

Keywords Regenerative medicine • Cardiopoiesis • Cardiovascular disease • Stem cell • Target population • Disease severity • Marker • Precision medicine

Introduction

Heart failure is a leading cause of mortality and morbidity; it limits quality of life and imposes a major societal burden.[1] Ischemic heart disease underpins two-thirds of all systolic heart failure.[2] Extensive myocardial remodelling and chamber enlargement portend poor outcomes, and standard treatments are often insufficient in such patients.[3] Cardiac transplantation or destination mechanical circulatory support remains high-risk therapeutic options that are further limited by donor availability, patient eligibility, and cost.[4]

By targeting myocardial restoration, cell-based therapies are alleged paradigm-shifting alternatives.[5,6] Clinical trials document reassuring feasibility and safety yet inconsistent efficacy, ascribed in part to unpredictable potency of cell products and limited retention.[7,8] These shortcomings impede advancement into cardiovascular practice.

Strategies for cell therapy optimization[9] include myocardial priming to improve cell homing,[10] exploiting resident cell populations[11] or leveraging combined cell regimens.[12,13] Guided cardiopoiesis is a recent option that enhances the cardioreparative functionality of patient-derived mesenchymal stem cells (MSC) and induces a restorative response in failing hearts.[14] The cardiopoietic phenotype demonstrated promise in proof-of-concept studies[15] and in the Cardiopoietic Stem Cell Therapy in Heart Failure (C-CURE) clinical trial.[16]

The Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial was executed to validate the efficacy and safety of cardiopoietic cells delivered via an enhanced retention performance catheter[17] in a larger population with advanced symptomatic heart failure of ischemic aetiology.[18]

Methods

Study design

The CHART-1 study is a prospective, multicentre, randomized, sham-controlled, patient- and evaluator-blinded clinical trial. Investigators at 39 centres in Europe and Israel participated (*Figure 1* and Supplementary material online, Section 1). Ethics committee approvals were obtained for each participating centre. The CHART-1 trial was registered with clinicaltrials.gov (NCT01768702) and EudraCT (2011-001117-13). The study design has previously been described,[18] and the study protocol is provided in Supplement 2.

Patients

Eligible patients gave written informed consent prior to any study-related procedures. Patients were not compensated for participation except for travel expenses. Patients were ≥ 18 to < 80 years old with left ventricular ejection fraction (LVEF) ≤ 35% (locally interpreted echocardiograms were used for screening), ischemic heart failure without need for revascularization, heart failure hospitalization, or outpatient vasoactive heart failure therapy (e.g. vasodilators, positive inotropic agents, vasopressors or diuretics) within 12 months, in New York Heart Association (NYHA) class II or greater at screening, and with NYHA class III or IV or Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) class ≥ 4 within 12 months.[18] Guideline-directed medical therapy, a 6-min walk distance > 100 to ≤400 m and Minnesota Living with Heart Failure Questionnaire (MLHFQ) score > 30 were required. Acute coronary syndrome or percutaneous coronary intervention within 90 days, or coronary artery bypass graft surgery within 180 days were exclusions.[18] Eligible patients were scheduled for bone marrow harvest and MSC expansion.

Approximately 2 weeks after screening, bone marrow (~65–85 mL) was collected from the iliac crest and shipped to a central production

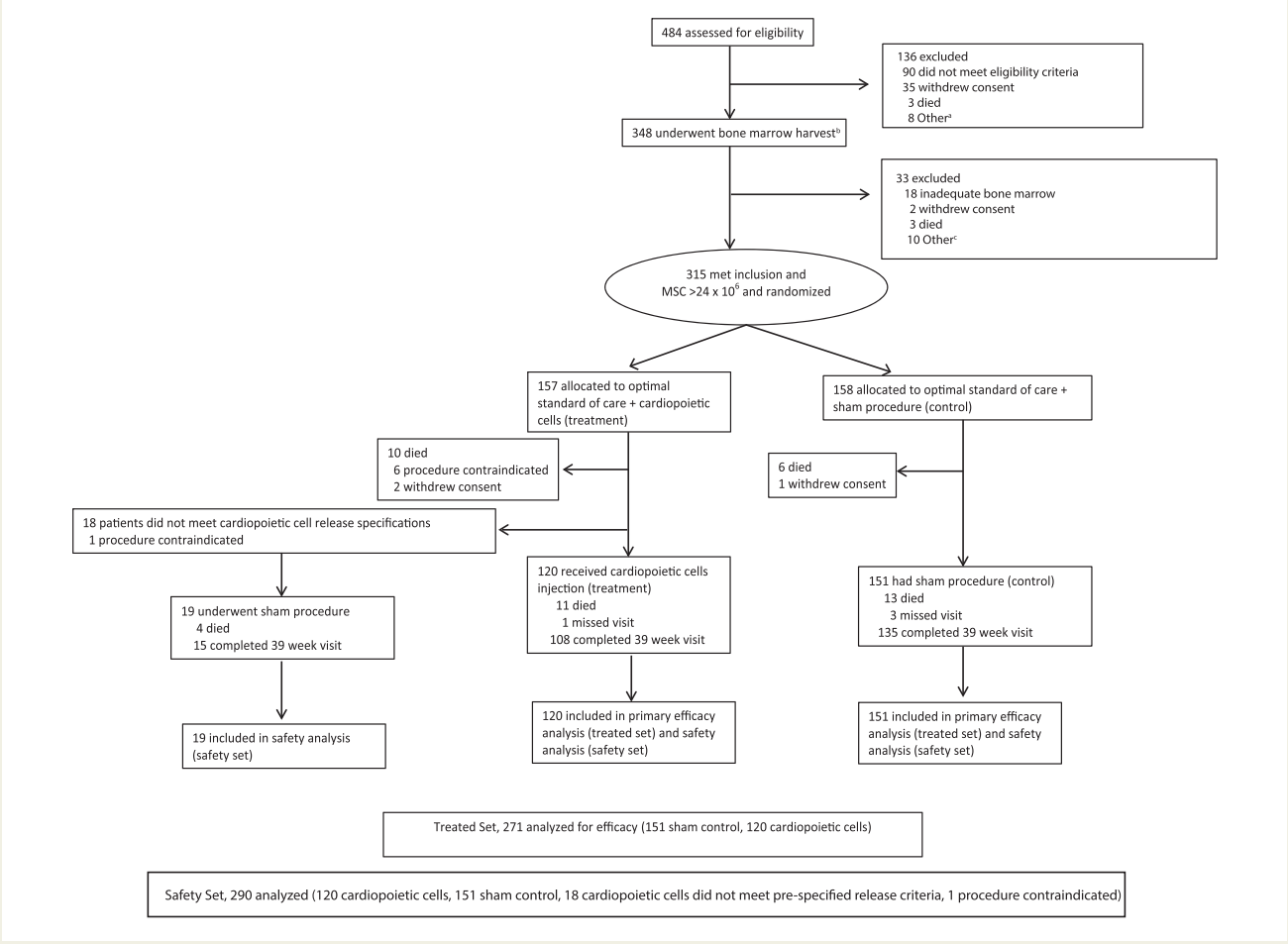

Figure 1 Consolidated standards of reporting trials diagram of the CHART-1 study. This figure depicts the patient flow through the trial. Eighteen (11.5%) patients randomized to active treatment and 7 (4.4%) patients randomized to control did not undergo the study procedure: 10 (6.4%) and 6 (3.8%) patients died, and 2 (1.3%) and 1 (0.6%) patients withdrew consent in the active and control groups, respectively. Six (3.8%) patients randomized to active treatment were discontinued because of procedural contraindications including left ventricular thrombus and aortic stenosis not identified at screening. Cell release specifications were not achieved in 18 (11.5%) patients randomized to active treatment; these patients and one additional patient for whom the injection procedure was deemed unsafe underwent a sham procedure and were followed separately. The remaining 120 patients underwent injection of cardiopoietic cells.
[a]Other reasons patients were withdrawn after screening but before bone marrow harvest included: withdrawal from the study by investigator or sponsor; patient missing or lost to follow-up; or other miscellaneous.
[b]Forty-eight (13.8%) patients who failed the first bone marrow harvest (1 due to inadequate sample volume, 8 due to improper harvesting or transport process, 21 because the sample was contaminated, and 18 because of inadequate expansion of MSCs) were eligible for a repeat harvest. Thirty-two patients underwent the second bone marrow harvest. Of the 16 who did not have a repeat, 5 were because the patient refused, 2 were due to SAEs (1 patient had a stroke and another was hospitalized for heart failure), and the rest for sponsor reason (either cell-process related or because study enrollment was nearing completion).
[c]Other reasons patients were withdrawn after bone marrow harvest but before randomization included: withdrawal from the study by sponsor or other miscellaneous.

facility (Celyad, Mont-Saint-Guibert, Belgium). If the bone marrow was of insufficient quantity, contaminated, or did not reach pre-specified cell production criteria, the harvest could be repeated. Patients with a second inadequate cell expansion or those who refused a second bone marrow harvest were discontinued from further participation.

Randomization and masking

Patients were randomized 1:1 to cardiopoietic cell injection or a sham control procedure after confirmation by the central production facility that > 24 million MSCs were achieved according to pre-specified release criteria. An Interactive Web Randomization Service was used according to a central randomization scheme (produced by Harvard Clinical Research Institute, Boston, Massachusetts) stratified by study centre with random permuted blocks within each centre. Patients and evaluators were blinded to study group assignment (eMethods in Supplementary ma terial online, Section 2.1).

Procedures

MSCs for patients randomized to active treatment were processed for lineage specification to derive cardiopoietic cells (eMethods in

Supplementary material online, Section 2.2).[16] Patients whose cell product did not meet release criteria because of inadequate identity, potency, content, purity, homogeneity, or microbiological content (eMethods in Supplementary material online, Section 2.2.2) received a sham procedure (*Figure 1*). Cryopreserved cardiopoietic cell batches meeting release criteria (C3BS-CQR-1 manufactured by Celyad, Mont-Saint-Guibert, Belgium) were shipped frozen to sites and reconstituted within 6 h before injection. Cardiopoietic cells were delivered using standard cardiac catheterization procedures and a cell retention-enhanced injection catheter (C-Cath$_{ez}$™; Celyad, Mont-Saint-Guibert, Belgium).[17] Intramyocardial injections (~0.5 mL each, ~1 cm apart) were made into left ventricle areas with wall thickness ≥ 8 mm, avoiding the apex and segments adjacent to the mitral or aortic valves. Target zones were mapped using biplane left ventricular angiography integrating preceding echocardiography information regarding wall thickness. A sham procedure, incorporating insertion of an introducer sheath, left ventricular angiography and pigtail catheter movements, was performed for patients randomized to the control group and in the patients whose cell product did not meet pre-specified release criteria.

Follow-up visits were conducted by the blinded investigator team.[18] Pre-procedure (baseline), 26 and 39-week echocardiograms were assessed centrally by a blinded core laboratory (eMethods in Supplementary material 1 online, Section 2.3).

Outcomes

The primary efficacy outcome was a hierarchical composite assessed at 39 weeks post-procedure comprising all-cause mortality (days to death), the number of worsening heart failure events (0, 1, or ≥ 2), MLHFQ score (≥ 10 point improvement, ≥ 10 point deterioration, or no meaningful change), 6-min walk distance (≥ 40 m improvement, ≥ 40 m deterioration, or no meaningful change), left ventricular end-systolic volume (LVESV) change (≥ 15 mL improvement, ≥ 15 mL deterioration, or no meaningful change), and LVEF change (≥ 4% absolute improvement, ≥ 4% absolute deterioration, or no meaningful change as assessed by the echocardiographic core laboratory).[18] Left ventricular assist device (LVAD) implantation or urgent heart transplantation was considered cardiac deaths for the efficacy analyses.

Safety assessment through Week 39 included all-cause mortality, rehospitalization, cardiac transplantation, myocardial infarction, stroke, aborted sudden death (resuscitated sudden death or appropriate implantable cardioverter defibrillator [ICD] shocks), and serious and non-serious adverse events.

Statistical analysis

The primary outcome was compared between groups using a Finkelstein-Schoenfeld approach,[19] which allows mortality and morbidity components to be combined with other important aspects of the heart failure disease process in one outcome. Patients are ranked with respect to their clinical outcomes through comparing every patient to every other patient on the hierarchy of component outcomes ordered by their relative importance[19] (eMethods in Supplementary material online, Section 2.4, e*Figure 1* in Supplementary material online, Section 2.4.1). Missing data are accommodated in the algorithm, with limited need for imputation (eMethods in Supplementary material online, Section 2.4; Supplement material 2 [protocol], page 40). The primary analysis was performed in patients as randomized, excluding patients whose cell product did not meet release criteria. Sensitivity analyses were performed in a modified intention-to-treat set including all patients who underwent the study procedure, and a per-protocol set excluding patients with major protocol deviations. All analyses were conducted using a two-sided significance level of 0.05.

A sample size of 120 patients per group was estimated to provide 87% power to detect a treatment effect corresponding to a Mann–Whitney estimator (the probability of a better response in the active treatment group plus half the probability of a tie) of 0.61 (values > 0.5 favour active treatment).[18] This treatment effect corresponds to a Mann–Whitney odds of 1.56, the relative probability of a better outcome on active treatment than on control. Homogeneity of the treatment effect across subgroups was assessed using chi-square tests computed from Mann–Whitney estimators and their corresponding variances in the subgroups. Post-hoc subgroup analyses evaluated treatment effect by baseline severity markers including left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), MLHFQ score, 6-min walk distance, and LVEF. Subpopulation Treatment Effect Pattern Plots (STEPPs) were used to evaluate the potential effect of treatment by baseline severity markers.[20]

Safety analyses included all patients according to the treatment received. Kaplan–Meier estimates of event rates through Week 39 and hazard ratios and 95% confidence intervals (CI) from Cox regression models are presented; groups were compared using log-rank tests.

Means ± standard deviation (SD) or median, and interquartile range (IQR), are presented for continuous variables, and geometric mean and 95% CI for log-transformed variables. Analyses are based on the data when the last patient to have a study procedure reached Week 39. SAS® (Cary, North Carolina, USA) version 9.3 was used for analyses.

A blinded Clinical Events Committee (CEC) adjudicated all deaths, worsening heart failure events, strokes, myocardial infarctions, and aborted sudden deaths from randomization (eMethods in Supplementary material online, Section 2.3). An independent interventional cardiologist adjudicated the relatedness of peri-procedural serious adverse events to the injection catheter, the catheterization procedure, and the cardiopoietic cell product. An independent Data Safety Monitoring Board (DSMB) reviewed ongoing safety reports, evaluated safety of the delivery device when the first 46 patients had 4 weeks follow-up, and reviewed safety data and a futility analysis when 120 patients had at least 13 weeks follow-up (eMethods in Supplementary material online, Section 2.3).

Results

Study population

Screening began 18 December 2012 and the last injection procedure (cardiopoietic cell therapy or sham) was performed 31 July 2015. A total of 484 patients provided informed consent to undergo eligibility screening, and 348 underwent bone marrow harvest (*Figure 1*). Adequate MSC expansion was achieved in 315 patients, and they were randomized to cardiopoietic cell therapy ($n = 157$) or sham control procedure ($n = 158$). Of these, 120 patients underwent injection of cardiopoietic cells and 151 had the sham procedure (*Figure 1*).

Baseline characteristics were well-balanced between the groups (*Table 1*). The mean ± SD age was 61.9 ± 8.6 years, 89.7% were men, and all were white. Eighty-five percent of patients had been hospitalized for heart failure within the previous year, and 21.8% were in NYHA Class II at screening. The mean ± SD centrally-assessed LVEF was 27.9 ± 7.0%. Patients were well-treated with guideline-directed medical and device therapy (*Table 1*) that remained consistent during follow-up (e*Figure 2* in Supplementary material online, Section 3.1).

The mean ± SD time between randomization and the study procedure was 59.8 ± 21.6 and 53.9 ± 11.7 days in patients randomized to and who received the active treatment vs. the sham procedure,

Table I Baseline characteristics

	Cardiopoietic cell treatment $n = 120$	Sham control $n = 151$
Demographics		
Male sex	107 (89.2)	136 (90.1)
Age (years)	61.6 (8.6)	62.1 (8.7)
Caucasian race	120 (100)	151 (100)
BMI (kg/m^2)	28.2 (3.7)	28.6 (4.4)
Heart Failure History		
NYHA class at screening		
I	0	0
II	23 (19.2)	36 (23.8)
III	96 (80)	114 (75.5)
IV	1 (0.8)	1 (0.7)
Time from first heart failure diagnosis to screening (months)	44.1 (12.3–100.1)	46.3 (16–97.7)
Heart failure hospitalization within 12 months	102 (85.0)	128 (84.8)
Number of heart failure hospitalizations in past 12 months	1.3 (0.8)	1.2 (0.5)
Comorbidities		
Chronic angina	38 (31.7)	56 (37.1)
CCSC-I	14 (11.7)	12 (7.9)
CCSC-II	20 (16.7)	36 (23.8)
CCSC-III	4 (3.3)	7 (4.6)
CCSC-IV	0	0
Percutaneous coronary intervention	98 (81.7)	103 (68.2)
Coronary artery bypass surgery	32 (26.7)	44 (29.1)
Myocardial infarction	106 (88.3)	133 (88.1)
Cerebrovascular atherosclerotic disease	13 (10.8)	13 (8.6)
Peripheral vascular disease	5 (4.2)	10 (6.6)
Atrial fibrillation	31 (25.8)	32 (21.2)
Atrial flutter	4 (3.3)	5 (3.3)
Sustained ventricular tachycardia	12 (10.0)	25 (16.6)
Ventricular fibrillation	10 (8.3)	20 (13.2)
ICD/AICD	46 (38.3)	63 (41.7)
CRT	25 (20.8)	25 (16.6)
Transplant list	1 (0.8)	0
Diabetes mellitus	45 (37.5)	71 (47)
Current smoking	12 (10)	25 (16.6)
Current alcohol abuse	4 (3.3)	7 (4.6)
Hypertension	99 (82.5)	124 (82.1)
Hypercholesterolemia	97 (80.8)	129 (85.4)
Renal impairment	25 (20.8)	36 (23.8)
Chronic lung disease	15 (12.5)	19 (12.6)
Baseline Therapies		
Baseline concomitant medications		
ACE inhibitor	96 (80)	117 (77.6)
ARB	14 (11.7)	21 (13.9)
ACE inhibitor or ARB	109 (90.8)	137 (90.7)
Beta blocker	107 (89.2)	135 (89.4)
CCB	6 (5)	27 (17.9)
Alpha blocker	36 (30)	39 (25.8)
MRA	94 (78.3)	109 (77.2)
Loop diuretic	104 (86.7)	123 (81.5)
Statin	107 (89.2)	125 (82.8)
Aspirin	76 (63.3)	100 (66.2)
Vitamin K antagonist	42 (35.0)	60 (39.7)
Baseline Vital Signs, Left Ventricular Parameters, and Biomarkers		
HR (bpm)	70.9 (12.5)	70.8 (10.3)
SBP (mmHg)	117 (14.4)	122.6 (15.3)
DBP (mmHg)	72.6 (8.5)	74.2 (10.3)
MLHFQ total score	48.8 (39.8–64.8)	46.5 (37–60)
6-min walk distance (meters)	332.5 (282–366.8)	332.5 (282.5–367.0)
LVESV (mL)	172.6 (140.4–224.2)	177.9 (133.3–212.4)
LVEF (%)	27 (23–32)	28 (24–32)
LVEDV (mL)	239.9 (197.4–294)	246.4 (198.2–285.6)
NT-proBNP (pg/mL)	1083.1 (450–2648.1)	1077.6 (483.7–2260.6)
SCr (μmol/L)	102.5 (85–128.6)	103 (86–128)
BUN (mmol/L)	7.6 (5.9–10.5)	7.5 (5.5–10.7)
eGFR (mL/min/1.73m^2)	60 (52–74.2)	60 (52–78)

Data are expressed as number (percent), mean (standard deviation), or median (interquartile range). There were no significant differences in baseline characteristics between groups ($P > 0.05$), except for history of percutaneous coronary intervention, calcium channel blocker use, and systolic blood pressure ($P < 0.05$). ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; bpm, beats per minute; BUN, blood urea nitrogen; CCB, calcium channel blocker; CCSC, Canadian Cardiovascular Society Classification; CRT-D, cardiac resynchronization therapy with defibrillator; CRT-P, cardiac resynchronization therapy with pacemaker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; ICD, automatic implantable cardioverter defibrillator; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MLHFQ, Minnesota Living with Heart Failure Questionnaire; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; SBP, systolic blood pressure; SCr, serum creatinine.

respectively. The median duration was 112.0 (IQR 78.0–157.5) minutes for the injection procedure and 36.0 (IQR 17.0–66.0) minutes for the sham procedure. The treatment group received a median of 19 (IQR 17–20) injections with a median injection volume of 9.6 (IQR 8.5–10) mL.

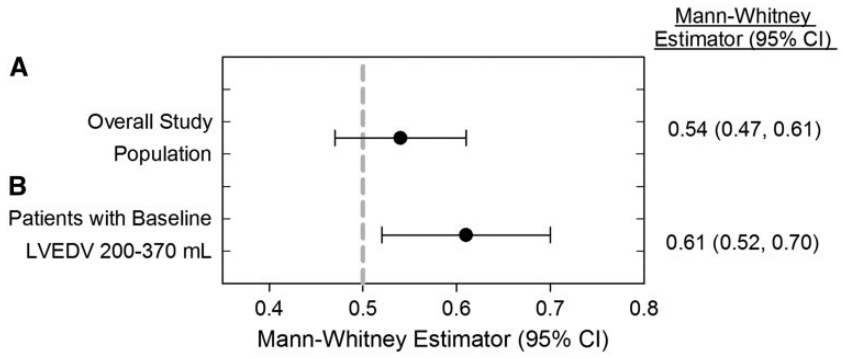

Figure 2 Primary efficacy outcome. Panel A depicts the primary efficacy outcome in the total population. The Mann–Whitney estimator, or the probability that the treatment group had a better outcome on the composite primary endpoint, was 0.54 (95% CI 0.47–0.61), $P = 0.27$ (a value > 0.5 favours the active treatment). The corresponding Mann–Whitney odds was 1.17 (95% 0.89–1.55). Panel B depicts the primary efficacy outcome in the subgroup of patients with LVEDV 200–370 mL. The Mann–Whitney estimator, or the probability that the treatment group had a better outcome on the composite primary endpoint, was 0.61 (95% CI 0.52–0.70), $P = 0.015$ (a value > 0.5 favours the active treatment). The corresponding Mann–Whitney odds was 1.57 (95% 1.09–2.35).

Primary end-point

The hierarchical composite primary endpoint across the total study cohort was neutral (Mann–Whitney estimator 0.54, 95% CI 0.47–0.61, $P = 0.27$) (*Figure 2*, panel A, e*Table 1* in Supplementary material online, Section 2.4.2), corresponding to a Mann–Whitney odds of 1.17 (95% CI 0.89–1.55). No significant between-group differences were noted for individual components of the primary outcome, but a signal for a benefit was observed across the categories of change in 6-min walk distance ($P = 0.07$) (*Table 2*).

Subgroup analysis

The response was similar according to sex (homogeneity $P = 0.43$), age (homogeneity $P = 0.25$), NYHA class (homogeneity $P = 0.69$), and geographic region (homogeneity $P = 0.71$). The effect of active treatment according to baseline heart failure severity was examined in post-hoc, exploratory analyses. A suggestion of efficacy for the active treatment was noted in patients with LVEDV, LVESV, or MLHFQ score greater than the median, and in those with 6-min walk distance less than the median (*Figure 3*). Subpopulation treatment effect pattern plots were used to further explore the pattern of treatment effect on the composite primary endpoint as a function of increasing, overlapping intervals of severity markers. The observed response patterns are shown in *Figure 4*. Patients with baseline LVEDV 200–370 mL receiving cardiopoietic cell treatment had a greater probability of a better outcome on the composite primary endpoint compared to the sham control group (Mann–Whitney estimator 0.61, 95% CI 0.52–0.70, $P = 0.015$; Mann–Whitney odds 1.57, 95% CI 1.09–2.35) (*Figure 2*, panel B). Patients with baseline LVEDV 200–370 mL (cardiopoietic cell therapy $n = 66$, sham control $n = 96$) treated with cardiopoietic cell therapy had a greater improvement in MLHFQ score from baseline that was of nominal significance. A greater absolute proportion had improvement in 6-min walk distance compared to sham control, and the absolute proportion with LVESV improvement was greater and with deterioration lesser in the active treatment vs. the sham control group, but these differences were not significant (*Table 2*). All components of the composite, including all-cause mortality and worsening heart failure events, were directionally consistent (*Table 2*).

Safety

Of the 120 patients undergoing study injections, 106 were without incident and 14 experienced catheter-procedure related serious adverse events. Of the 14 patients, each developed one of the following: ventricular tachyarrhythmia (4 sustained, responsive to cardioversion), left bundle branch block (3 sustained, 2 receiving cardiac resynchronization therapy [CRT] for persistent heart failure 2 and 4.5 months after injection, respectively), dissection of the ascending aorta requiring surgery (in a patient with known calcific and dilated thoracic aorta, occurring prior to the procedure while catheter was introduced), transient ischemic attack (1 with aphasia; normal head computerized tomography [CT] scan and resolution by 48 h), femoral artery stenosis (1 with claudication; sub-total occlusion at site of closure device implantation, managed medically), and pericardial effusion (4, three with tamponade responsive to drainage, 1 without hemodynamic consequences resolving spontaneously). No cases of pericardial tamponade occurred in the final 72 active cases after additional procedural training and oversight.

Cardiac markers (CK-MB and high-sensitivity cardiac troponin T [hs-cTnT]) were increased at 6 and 24 h following the cell injection procedure. At 6 h, CK-MB had increased a median of 3.35 (IQR -0.600–59.800) μg/L in the active treatment group, compared to a median change of -0.20 (IQR -11.200–8.800) μg/L in the control group. At 24 h, median changes were 0.90 (IQR 0.1–1.8) and -0.30 (IQR -0.80–0.00) μg/L in patients treated with active and sham control, respectively, a ratio of 2.08 ($P < 0.001$). The median change in hs-cTnT at 6 h was 0.088 (IQR 0.04–0.151) μg/L in the cardiopoietic cell treatment group and 0.001 (IQR -0.002–0.003) μg/L in the sham control. At 24 h, the median change from baseline was 0.059 and

Table 2 Components of the primary efficacy endpoint in the total cohort and subpopulation with elevated left ventricular end-diastolic volume

	Total cohort $n = 271$				Patients with baseline left ventricular end-diastolic volume 200–370 mL $n = 162$			
	Cardiopoietic cell treatment $n = 120$	Sham control $n = 151$	HR or M-W odds (95% CI)	*P*-value	Cardiopoietic cell treatment $n = 66$	Sham control $n = 96$	HR or M-W odds (95% CI)	*P*-value
All-cause mortality through Week 39 (Kaplan–Meier %)	11 (9.2)	12 (7.9)	1.18 (0.52, 2.67)	.70	3 (4.5)	6 (6.2)	0.73 (0.18, 2.93)	0.66
Worsening heart failure events								
0	100 (83.3)	128 (84.8)			58 (87.9)	79 (82.3)		
1	11 (9.2)	14 (9.3)	1.03 (0.87, 1.23)	.72	4 (6.1)	9 (9.4)	0.90 (0.71, 1.12)	0.34
≥2	9 (7.5)	9 (6)			4 (6.1)	8 (8.3)		
Change in MLHFQ total score from baseline	[a]*n* = 108	[a]*n* = 136			[a]*n* = 63	[a]*n* = 89		
≥10-point improvement (decrease)	64 (59.3)	66 (48.5)			43 (68.3)	44 (49.4)		
No meaningful change	37 (34.3)	60 (44.1)	0.84 (0.68, 1.04)	.12	15 (23.8)	39 (43.8)	0.74 (0.55, 0.99)	0.04
≥10-point deterioration (increase)	7 (6.5)	10 (7.4)			5 (7.9)	6 (6.7)		
Change in 6-min walk distance from baseline	[a]*n* = 108	[a]*n* = 131			[a]*n* = 63	[a]*n* = 85		
≥40 m improvement (increase)	50 (46.3)	40 (30.5)			27 (42.9)	21 (24.7)		
No meaningful change	39 (36.1)	69 (52.7)	0.82 (0.66, 1.02)	.07	25 (39.7)	51 (60)	0.79 (0.59, 1.06)	0.12
≥40 m deterioration (decrease)	19 (17.6)	22 (16.8)			11 (17.5)	13 (15.3)		
Change in LVESV from baseline	[a]*n* = 102	[a]*n* = 124			[a]*n* = 63	[a]*n* = 85		
≥15 mL improvement (decrease)	51 (50)	56 (45.2)			36 (57.1)	41 (48.2)		
No meaningful change	33 (32.4)	36 (29)	0.89 (0.73, 1.09)	.26	18 (28.6)	23 (27.1)	0.81 (0.60, 1.09)	0.17
≥15 mL deterioration (increase)	18 (17.6)	32 (25.8)			9 (14.3)	21 (24.7)		
Change in LVEF from baseline	[a]*n* = 102	[a]*n* = 124			[a]*n* = 63	[a]*n* = 85		
≥4% absolute improvement (increase)	69 (67.6)	82 (66.1)			42 (66.7)	56 (65.9)		
No meaningful change	28 (27.5)	33 (26.6)	0.97 (0.81, 1.15)	.73	19 (30.2)	22 (25.9)	0.96 (0.74, 1.25)	.76
≥4% absolute deterioration (decrease)	5 (4.9)	9 (7.3)			2 (3.2)	7 (8.2)		

Data are expressed as number (percent). Hazard ratio (HR) for active treatment vs. control is provided for mortality. The Mann–Whitney (M-W) odds for *worse* outcome in active vs. control is given for ordered categories. Values <1.0 favour active treatment.
LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MLHFQ, Minnesota Living with Heart Failure Questionnaire.
[a]*n* reflects the number of patients with data.

0.001 μg/L in the active treatment and sham control group patients, respectively ($P < 0.001$). At Week 39, hs-cTnT levels were comparable: the median change was 0.001 μg/L in both groups, treatment ratio 1.014, 95% CI 0.901–1.142.

Adjudicated clinical endpoints and investigator-reported adverse events through Week 39 according to the actual treatment received (i.e. the 19 patients randomized to active who received a sham procedure are included in the sham group) are shown in *Table 3*. Similar proportions of patients died who underwent the active treatment and sham procedure (HR 1.02, 95% CI 0.45–2.29). The adjudicated causes of death were similar, although sudden cardiac death occurred in no patient in the cardiopoietic cell treatment group and in 4 patients who underwent the sham procedure. Aborted or sudden cardiac death occurred in 1 (0.9% [Kaplan–Meier estimated risk]) cardiopoietic cell treatment patient and 9 (5.4%) of sham control patients (HR 0.16, 95% CI 0.02–1.23, $P = 0.04$). One patient who received active

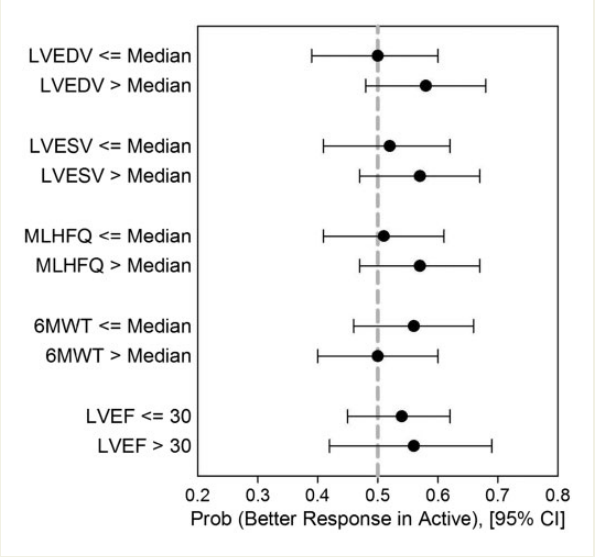

Figure 3 Primary efficacy outcome by markers of disease severity. This figure shows the Mann–Whitney estimator, or the probability that the treatment group had a better outcome on the composite primary endpoint, for patients above and below the median values for LVEDV, LVESV, MLHFQ score, and 6-min walk distance, and for LVEF above and below a cutpoint of 30%.

treatment had a cardiac transplantation. Occurrence of other adjudicated clinical endpoints including myocardial infarction and stroke were similar in the groups (*Table 3*). Similar hospitalization rates after the index procedure were observed through Week 39 (26.2% for active treatment vs. 27.4% for control, HR 1.01, 95% CI 0.64–1.59, P = 0.96), most often due to heart failure (15.2% for active and 18.1% for control) (e*Table 2* in Supplementary material online, Section 3.2).

Discussion

The CHART-1 study is the largest cardiovascular regenerative medicine trial to date addressing the effect of cardiopoiesis-based cell therapy in ischemic heart failure patients with moderate to severe symptoms. In this at-risk population with limited therapeutic options, the trial was neutral regarding the primary endpoint, a hierarchical composite encompassing all-cause mortality, worsening heart failure events, MLHFQ score, 6-min walk distance, LVESV and LVEF assessed at 39 weeks. Exploration of the primary composite endpoint according to baseline heart failure severity revealed a clinically relevant population that appeared to benefit from cardiopoietic cell therapy. This sizable target population, representing 60% of the whole study cohort, was characterized by severe heart enlargement (baseline LVEDV 200–370 mL). These patients had greater improvement in 6-min walk distance consistent with favourable effects on myocardial structure (i.e. LVESV). Directionally similar treatment effects on all-cause mortality and worsening heart failure events were also observed in this subset. Patients displaying a lower (< 200 mL) or greater (> 370 mL) LVEDV did not appear to respond to cell therapy in this study. These data suggest that targeted patient selection using disease severity markers should be considered for future clinical trials and/or potential clinical application of cell therapy in patients with heart failure. Indeed, a call for a focus on precision medicine has been issued, where clinical studies would target well-defined patient populations to improve development of effective cardiovascular treatments.[21]

The CHART-1 study corroborates, in a larger heart failure cohort, the feasibility, safety and initial efficacy signals detected in the C-CURE trial.[16] Clinical surveillance documented safety through 39 weeks without excess adverse events attributable to cardiopoietic cell therapy. Peri-procedural events in the CHART-1 trial were consistent with well-established complications of left heart catheterization and/or intramyocardial injection, which can spike with the introduction of a new device but generally recede as interventional experience and procedural volume accrue.[22] Notably, a significantly lower incidence of sudden or aborted sudden deaths was documented in cardiopoietic cell-treated patients compared to controls, underpinning clinical safety across both C-CURE[16] and CHART-1 trials.

Heart failure clinical trial experience points to inter-trial and inter-patient variability in cell therapy outcomes.[23–25] Recognizing that only a limited number of patients with ischemic heart disease harbors reparative stem cells, processes have been introduced to optimize reparative outcome.[26] The ixCELL-DCM trial is a recent example where a reduction in adjudicated clinical cardiac events in ischemic cardiomyopathy was documented after delivery of an expanded multicellular product.[13] Leveraging the cardiopoiesis platform in conjunction with a novel catheter fitted with a curved needle delivery system,[15] the CHART-1 clinical experience advances current knowledge by identifying disease severity as a potential modifier of cell therapy benefit.

In this regards, heart failure is a progressive and heterogeneous syndrome where conventional symptoms or ejection fraction often fail to identify patients that optimally respond to a therapy. Non-uniform responses in advanced heart failure have been reported in a spectrum of therapies including revascularization,[27] interventions targeting functional mitral regurgitation[28] or CRT.[29] Of note and consistent with the CHART-1 findings, the degree of baseline LV enlargement has previously been detected as a modifying factor influencing therapeutic responsiveness to patients undergoing CRT, where the response was most robust in patients with LV end-diastolic volume index >125 ml/m^2.[29] The relationship between left ventricular volumes and clinical outcomes in heart failure is well recognized.[3] The CHART-1 study extends these findings by defining a range of LVEDV that appeared to segregate heart failure patients with the highest potential to benefit from cell-based therapy. Evidence from the CHART-1 experience, in the context of prior knowledge with other therapies[27–29] and recent proposals to streamline clinical development,[21] suggests that heart failure management should be patient-tailored based on disease severity markers, such as degree of left ventricular dilation.

The present data should be interpreted in the context of the following limitations. Use of a composite primary endpoint was intended to increase the statistical precision of the trial, yet if an important component of the composite outcome is not substantially modified by the treatment then the statistical power to detect effects on the overall composite may be reduced.[30] Indeed, neutrality in the primary hierarchical composite endpoint within the overall study population was related primarily to a neutral effect on all-cause mortality or worsening heart failure. This finding may reflect the 39-week

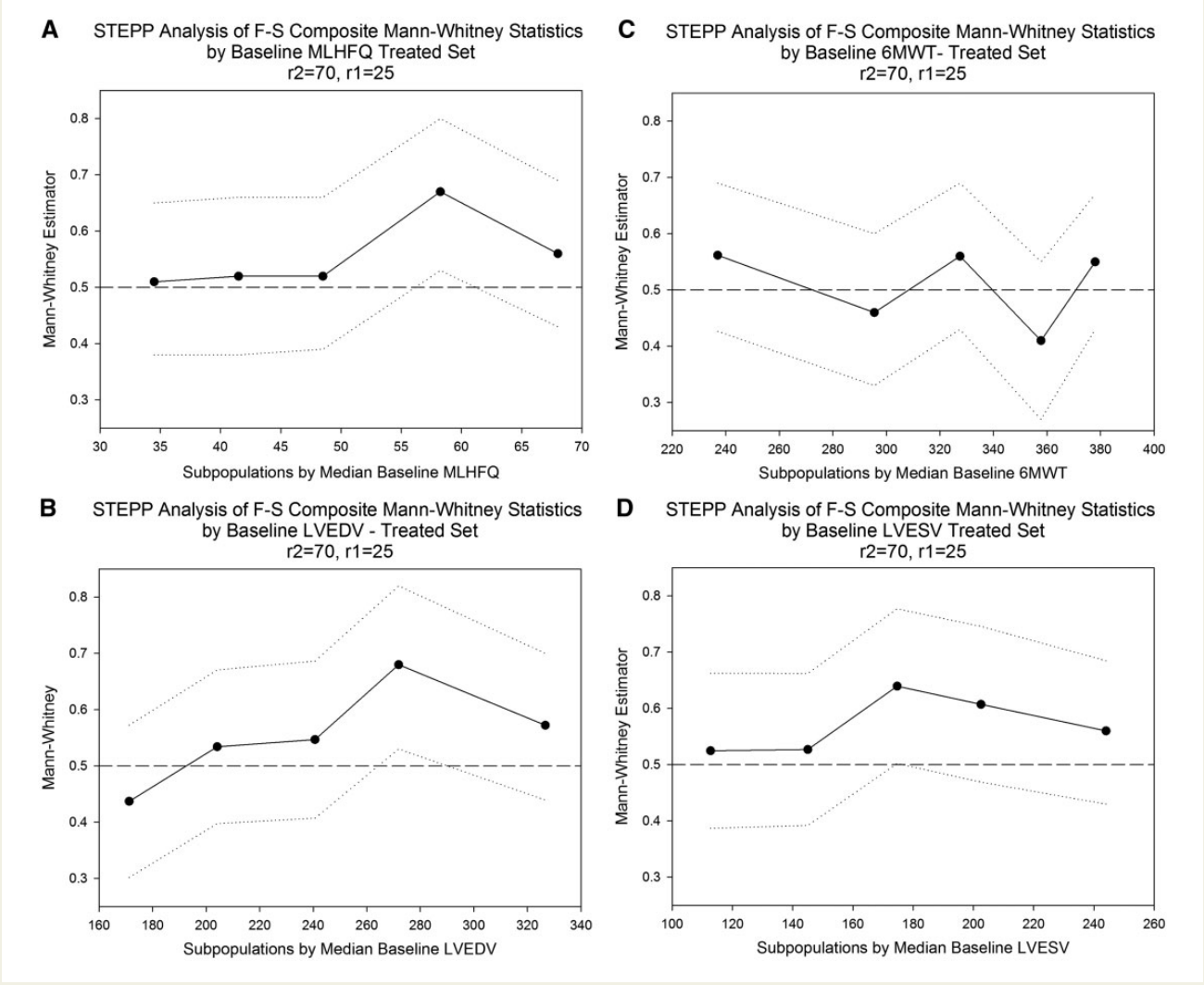

Figure 4 Subpopulation treatment effect pattern plot by markers of disease severity. Subpopulation Treatment Effect Pattern Plots (STEPPs) were used to further evaluate the potential effect of treatment according to baseline markers of disease severity. This figure shows the STEPP results according to baseline MLHFQ score (panel A), baseline LVEDV (panel B), baseline 6-min walk distance (panel C), and baseline LVESV (panel D).

time point for the primary outcome. In this context, longer follow-up is planned to evaluate the effect of cardiopoietic cell therapy. Eighteen patients initially randomized to the cardiopoietic cell group did not meet cardiopoietic cell release criteria and the procedure was contraindicated in one patient; these patients received a sham procedure and were not included in the primary efficacy analysis. This approach assesses the effect of cardiopoietic cells in those patients who actually received them. The result of an analysis in a modified intent-to-treat set, which included the process failures who underwent a sham procedure in the active group for analysis, was nearly identical (Mann–Whitney estimator 0.54, $P = 0.283$) to that of the primary endpoint (Mann–Whitney estimator 0.54, $P = 0.27$). STEPP was used to identify the influence of LVEDV on the primary endpoint. This approach is methodologically preferred compared to conventional post-hoc analysis, as STEPP constructs overlapping subpopulations along the continuum of the covariate, improving the precision of the estimated treatment effects.[31] LV volumes and function were assessed by transthoracic echocardiography using established guidelines.[18] To minimize reproducibility issues, measurements were performed by a trained single echocardiographer per center with central core analyses. Although inadequate bone marrow aspiration or suboptimal outcome of the production process preventing cell product release occurred, these are expected to diminish as the technology and procedural experience matures. Finally, the study population was Caucasian and predominantly male. The present findings should be confirmed in subsequent studies with broader representation of women and non-Caucasian racial groups.

Conclusions

The CHART-1 study is the largest cardiovascular regenerative medicine clinical trial to date that addresses the efficacy and safety of cardiopoiesis-based cell therapy in ischemic heart failure. The trial

Table 3 Mortality and cardiovascular events and adverse events through 39 weeks[a]

	Cardiopoietic cell treatment $n = 120$	Sham control $n = 170$[b]
Total deaths[c]	10 (8.3)	14 (8.2)
During hospitalization for study procedure		
Cardiovascular – other CV cause	1 (0.8)	0
After hospitalization for study procedure		
Cardiovascular death	9 (7.6)	12 (7.1)
Heart failure/cardiogenic shock	6 (5.0)	7 (4.2)
Sudden cardiac death	0	4 (2.4)
Acute MI	1 (0.9)	0
Stroke	1 (0.9)	0
Undetermined cause	1 (0.9)	1 (0.6)
Non-cardiovascular death	0	2 (1.2)
Infection	0	2 (1.2)
Non-fatal events		
Cardiac transplantation	1 (0.9)	0
Myocardial infarction		
During hospitalization for study	0	0
After hospitalization for study	1 (0.9)	1 (0.6)
Stroke		
During hospitalization for study	1 (0.8)	0
After hospitalization for study	2 (1.8)	2 (1.2)
Aborted sudden cardiac death		
During hospitalization for study	0	0
After hospitalization for study	1 (0.9)	5 (3.0)
Adverse Events Reported by interventional investigators (not blinded)		
Any AE	25 (20.8)	9 (5.3)
AE related to cardiopoeitic cells or sham as reported by investigator	10 (8.3)	2 (1.2)
AE related to the catheter as reported by investigator	12 (10)	1 (0.6)
Any serious AE	17 (14.1)	3 (1.8)
Serious AE with fatal outcome	2 (1.7)	0
Adverse Events Reported by evaluator investigators (blinded)		
Any AE	62 (52.5)	90 (53.0)
AE related to cardiopoietic cells or sham as reported by investigator	5 (4.2)	2 (1.2)
AE related to the catheter as reported by investigator	4 (3.4)	2 (1.2)
Any serious AE	44 (37.2)	63 (37.1)
Serious AE with fatal outcome	8 (6.8)	17 (10)

Data are expressed as number (percent).
AE, adverse event; CV, cardiovascular; MI, myocardial infarction.
[a]Kaplan–Meier %.
[b]Safety set comprised of 151 sham control and 19 patients who did not meet cardiopoietic cell release specifications or had a contraindication but underwent sham procedure.
[c]Note the number of deaths shown in *Figure 1* is different from the values shown in this table, because *Figure 1* includes 4 deaths (1 in patients randomized to and treated with active, 2 randomized to and treated with sham, and 1 randomized to active and treated with sham) who died after day 273 but before a Week 39 visit could be performed. Thus, they are included in the patient disposition figure based on visit completion, but they are not included in calculation of Week 39 (or day 273) event rates. There were a total of 24 deaths by day 273: 10 in patients randomized to and treated with active, 11 randomized to and treated with sham, and 3 randomized to active and treated with sham. There were 2 additional patients who had an urgent LVAD placed, but who did not die by day 273: 1 patient randomized to and treated with active, and 1 patient randomized to and treated with sham. These urgent LVAD placements were considered deaths in the efficacy analyses.

was neutral regarding the primary endpoint. Using markers of heart failure severity, the CHART-1 trial identified a clinically relevant patient population characterized by severe heart enlargement (LVEDV 200–370 mL) that appeared to derive consistent benefit from cardiopoietic cell treatment as regards the primary endpoint. Insights from the CHART-1 trial, namely targeting the patient population using indices of disease severity, should be considered for cardiopoietic cell therapy in future clinical trials. This application of the CHART-1 results could be an effective step towards cell-based precision medicine in patients with advanced ischemic heart failure.[32]

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Dr. Seron: Employee of Celyad.

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