ORIGINAL RESEARCH ARTICLE

Cardiopoietic stem cell therapy in ischaemic heart failure: long-term clinical outcomes

Jozef Bartunek[1]*, Andre Terzic[1,2]*, Beth A. Davison[3], Atta Behfar[2], Ricardo Sanz-Ruiz[4], Wojciech Wojakowski[5], Warren Sherman[6], Guy R. Heyndrickx[1], Marco Metra[7], Gerasimos S. Filippatos[8], Scott A. Waldman[9], John R. Teerlink[10,11], Timothy D. Henry[12], Bernard J. Gersh[2], Roger Hajjar[13], Michal Tendera[5], Stefanie Senger[3], Gad Cotter[3], Thomas J. Povsic[14], William Wijns[15] and for the CHART Program

[1]Cardiovascular Center, OLV Hospital, Moorselbaan 164, Aalst, B-9300, Belgium; [2]Department of Cardiovascular Medicine, Mayo Clinic, Center for Regenerative Medicine, 200 First Street SW, Rochester, MN 55905, USA; [3]Momentum Research, Inc., Durham, NC, USA; [4]Cardiology Department, Hospital General Universitario Gregorio Marañón and CIBERCV (Instituto de Salud Carlos III), Madrid, Spain; [5]Department of Cardiology and Structural Heart Disease, Medical University of Silesia, Katowice, Poland; [6]Consultant, South Egremont, MA, USA; [7]Cardiology, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University and Spedali Civili, Brescia, Italy; [8]National and Kapodistrian University of Athens, School of Medicine, Attikon University Hospital, Athens, Greece; [9]Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA; [10]School of Medicine, University of California San Francisco, San Francisco, CA, USA; [11]Section of Cardiology, San Francisco Veterans Affairs Medical Center, San Francisco, CA, USA; [12]The Carl Edyth Lindner Center for Research and Education at The Christ Hospital, Cincinnati, OH, USA; [13]Phospholamban Foundation, Amsterdam, Netherlands; [14]Duke Clinical Research Institute and Duke University Medical Center, Durham, NC, USA; [15]The Lambe Institute for Translational Medicine and Curam, National University of Ireland Galway and Saolta University Healthcare Group, Galway, Ireland

Abstract

Aims   This study aims to explore long-term clinical outcomes of cardiopoiesis-guided stem cell therapy for ischaemic heart failure assessed in the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial.

Methods and results   CHART-1 is a multinational, randomized, and double-blind trial conducted in 39 centres in heart failure patients ($n = 315$) on standard-of-care therapy. The 'active' group received cardiopoietic stem cells delivered intramyocardially using a retention-enhanced catheter. The 'control' group underwent patient-level sham procedure. Patients were followed up to 104 weeks. In the entire study population, results of the primary hierarchical composite outcome were maintained neutral at Week 52 [Mann–Whitney estimator 0.52, 95% confidence interval (CI) 0.45–0.59, $P = 0.51$]. Landmark analyses suggested late clinical benefit in patients with significant left ventricular enlargement receiving adequate dosing. Specifically, beyond 100 days of follow-up, patients with left ventricular end-diastolic volume of 200–370 mL treated with ≤19 injections of cardiopoietic stem cells showed reduced risk of death or cardiovascular hospitalization (hazard ratio 0.38, 95% CI 0.16–0.91, $P = 0.031$) and cardiovascular death or heart failure hospitalization (hazard ratio 0.28, 95% CI 0.09–0.94, $P = 0.040$). Cardiopoietic stem cell therapy was well tolerated long term with no difference in safety readouts compared with sham at 2 years.

Conclusions   Longitudinal follow-up documents that cardiopoietic stem cell therapy is overall safe, and post hoc analyses suggest benefit in an ischaemic heart failure subpopulation defined by advanced left ventricular enlargement on tolerable stem cell dosing. The long-term clinical follow-up thus offers guidance for future targeted trials.

Keywords   Cardiopoiesis; Clinical trial; Heart failure; Longitudinal; Regenerative medicine; Stem cell

Introduction

Heart failure of ischaemic origin is a prevalent cause of morbidity and mortality.[1,2] Patients with extensive cardiac remodelling and chamber enlargement have limited therapeutic options and exhibit poor long-term survival.[3] New therapies that would enhance standard regimens of care are thus warranted and have included regenerative options.[4,5]

Leveraging cardiopoiesis aimed at optimizing the reparative functionality of patient-derived stem cells,[6–10] along with a delivery device designed for increased cell retention,[11] the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial provided insights into next-generation biotherapies.[12] In the overall study population with prior myocardial infarction (MI), the trial was neutral on the primary endpoint consisting of a hierarchical composite of mortality, worsening heart failure, Minnesota Living with Heart Failure Questionnaire score, 6 min walk test, left ventricular (LV) end-systolic volume, and LV ejection fraction (LVEF) at 9 months of follow-up.[13] Exploring the primary endpoint according to disease severity at baseline revealed a clinically relevant subpopulation with an LV end-diastolic volume (LVEDV) of 200 to 370 mL that appeared to benefit from cardiopoietic cell therapy.[13] Insights at 1 year indicated reduction in LV volumes, with reverse remodelling most pronounced in patients receiving fewer injections.[14] Heart failure severity and treatment posology may thus bear on cell therapy outcome, and we report here the long-term outcomes, up to 2 year, post-cell therapy.

Methods

Patient population and treatment

In 39 centres, 315 patients with ischaemic heart failure and LVEF ≤35%, for whom adequate mesenchymal stem cells were obtained from bone marrow, were included in the CHART-1 trial to receive cardiopoietic stem cells or sham procedure.[12–14] The trial was registered with clinicaltrials. gov (NCT01768702) and EudraCT (2011-001117-13) and approved by regulatory bodies and ethics committees at each participating site. Participants on guidelines-directed standard of care, defined as the best attempted medical treatment with optimization of medical treatment implemented prior to enrolment, were randomized as follows: 157 to receive cardiopoietic stem cells delivered intramyocardially ('active' group) and 158 to receive sham procedure ('control' group). Of these, as described,[13] 120 active and 151 control patients received the assigned treatment while 19 patients assigned to active treatment received the sham control procedure. In the active group, up to 21 intramyocardial

injections using a retention-enhanced catheter (C-Cath$_{ez}$, Celyad) of 0.5 mL of cardiopoietic stem cells were delivered approximately 1 cm apart in the left ventricle.[12–15] The decision to stop injections was left at the discretion of the operator depending on the risk of clustering the injections or performing injections in zones of LV wall thickness <8 mm or zones with increased risk of perforation such as apical segments. The patient-level sham procedure involved placement of an introducer sheath, LV angiography, and pigtail catheter movements without intramyocardial injections.[12] Ethical considerations circumvented procedural risk related to placebo injections in a vulnerable population. Patients provided written, informed consent.

Assessments

High-sensitivity troponin T, with a reference range of 0–0.014 µg/L, was measured at baseline, 6, and 24 h following the procedure and analysed by a central laboratory. Patients were followed through Week 104 post-procedure by an assessor investigator blinded to study treatment with scheduled hospital visits at 4, 13, 26, 39, 52, and 104 weeks. The 2 years of follow-up visit was to be performed per protocol at Week 104 or up to 30 days thereafter.[12]

Endpoints

The hierarchical endpoint comprising all-cause mortality, worsening of heart failure events, and changes in Minnesota Living with Heart Failure Questionnaire total score, 6 min walk test distance, LV end-systolic volume, and LVEF was measured at 39 and 52 weeks post-procedure.[12,13] Other predefined endpoints evaluated at Weeks 52 and 104 included cardiovascular (CV) death or heart failure hospitalization, all-cause and CV mortality, and death or CV hospitalization.[12] Predefined safety endpoints included all-cause mortality through 104 weeks post-procedure; and all-cause and cause-specific hospitalizations, MI, stroke, aborted sudden death, and serious adverse events through 52 and 104 weeks post-procedure. Cause of death and worsening of heart failure, MI, stroke, and aborted sudden death through Week 104 were adjudicated by a blinded Clinical Events Committee.

Statistical analysis

Treatment groups were compared with respect to the primary hierarchical composite endpoint using the Finkelstein–Schoenfeld method[12,16]; treatment effect is expressed as a Mann–Whitney estimator, that is, the probability of a better outcome on active treatment, with a value >0.5 favouring cell therapy. Treatment groups were

compared with respect to time-to-event outcomes using log-rank tests; hazard ratios (HRs) and associated 95% confidence intervals (CIs) were estimated from Cox regression models. Post hoc analyses evaluated treatment effects in two subgroups of patients identified post hoc as potentially benefitting from cell therapy: patients with baseline LVEDV of 200–370 mL and active patients who received ≤19 injections.[13,14] Landmark analyses were performed post hoc to explore early relative to later hazards and benefits associated with therapy. As there is a limited understanding of the recovery time course following an interventional procedure delivering a biologic therapy, landmarks were chosen for each endpoint based on the first-time hazards crossed in the two groups rather than any pre-specified time point. For the purpose of efficacy analysis, an urgent implantation of an LV assist device or heart transplantation was considered a CV death. The log-transformed maximum change from baseline of the two post-procedure 6 and 24 h of troponin T measures was compared among patients grouped by number of injections. Safety analyses were conducted in patients according to treatment received (safety set) and efficacy analyses in patients treated as randomized (treated set). Two-sided $P < 0.05$ was considered statistically significant. SAS® software Version 9.4 (SAS Institute, Cary, NC) was used for analyses.

Results

Composite endpoint at Week 52 post-cell therapy

From the 315 patients enrolled in the CHART-1 trial,[13] 271 were treated as randomized (treated set). In the overall patient population, results for the primary hierarchical composite endpoint were neutral at Week 52 (M–W estimator 0.52, 95% CI 0.45–0.59, $P = 0.51$) and similar compared with Week 39 (M–W estimator 0.54, 95% CI 0.47–0.61, $P = 0.27$; Figure 1). In patients with baseline LVEDV (200–370 mL), previously identified by exploratory analyses as a subgroup who may have derived benefit,[13,14] results at 52 weeks for the primary endpoint showed a sustained M–W estimator value in line with Week 39 (M–W estimator 0.60, 95% CI 0.51–0.69, $P = 0.024$ vs. M–W estimator 0.61, 95% CI 0.52–0.70, $P = 0.015$; Figure 1).

Long-term follow-up and landmark analyses

The median follow-up in the treated set was 734 days (104.9 weeks). Of the 271 patients treated as randomized, 26 (21.7%) cell-treated patients and 39 (25.9%) sham-treated patients died or had an urgent LV assist device or transplant (HR 0.84, 95% CI 0.51–1.38, $P = 0.49$, Figure 2). Estimated hazards for all-cause mortality first crossed in the two groups at ~1 year. Prior to 1 year, patients who received intramyocardial injection of cells were at numerically higher risk of death (HR 1.13, 95% CI 0.57–2.27, $P = 0.72$), while in those patients who survived to 1 year, the risk of death declined somewhat in cell treated compared with sham (HR 0.62, 95% CI 0.30–1.28, $P = 0.19$). Comparable landmark trends were observed in patients with a baseline LVEDV between 200 and 370 mL (HR 0.91, 95% CI 0.30–2.79, $P = 0.87$ prior to 1 year, HR 0.76, 95% CI 0.32–1.79, $P = 0.53$ beyond 1 year; Figure 2).

In further landmark analyses, estimated hazards for composite outcomes of death or CV hospitalization and CV death

Figure 1 Primary composite hierarchical endpoint at 39 and 52 weeks. The Mann–Whitney estimator, or the probability that the treatment group had a better outcome on the composite primary endpoint, and corresponding 95% confidence interval at 39 and 52 weeks in the entire patient population and in the subgroup of patients with LVEDV of 200–370 mL. The value >0.5 of Mann–Whitney estimator favours the active treatment. CI, confidence interval; LVEDV, left ventricular end-diastolic volume.
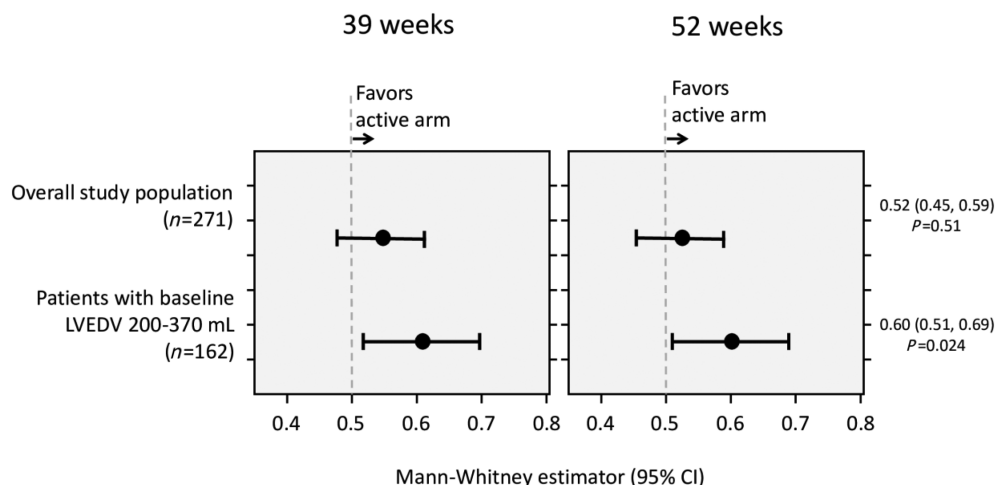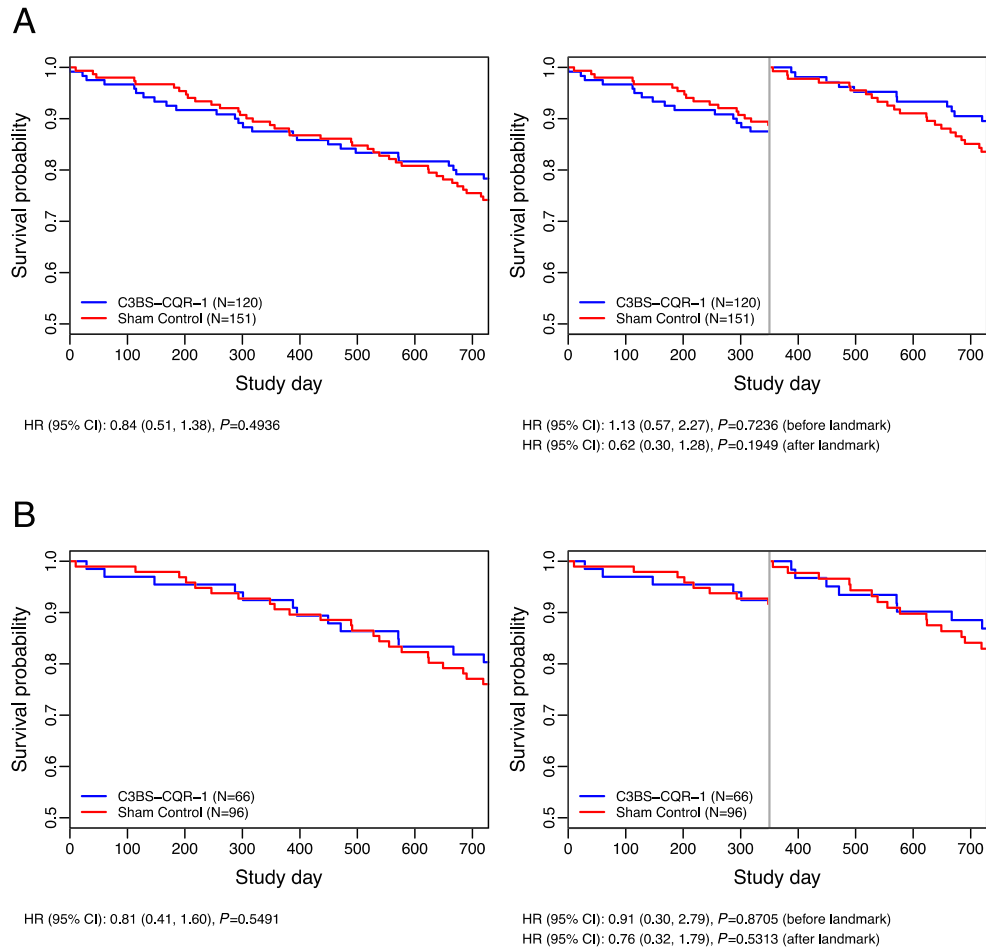
Figure 2 Landmark analyses of all-cause mortality. Kaplan–Meier estimates of all-cause mortality through 104 weeks, in patients treated as randomized (treated set) (left panel), with landmark analysis (right panel), in (A) all patients and (B) patients with left ventricular end-diastolic volume of 200–370 mL. CI, confidence interval; HR, hazard ratio.

A

HR (95% CI): 0.84 (0.51, 1.38), *P*=0.4936

HR (95% CI): 1.13 (0.57, 2.27), *P*=0.7236 (before landmark)
HR (95% CI): 0.62 (0.30, 1.28), *P*=0.1949 (after landmark)

B

HR (95% CI): 0.81 (0.41, 1.60), *P*=0.5491

HR (95% CI): 0.91 (0.30, 2.79), *P*=0.8705 (before landmark)
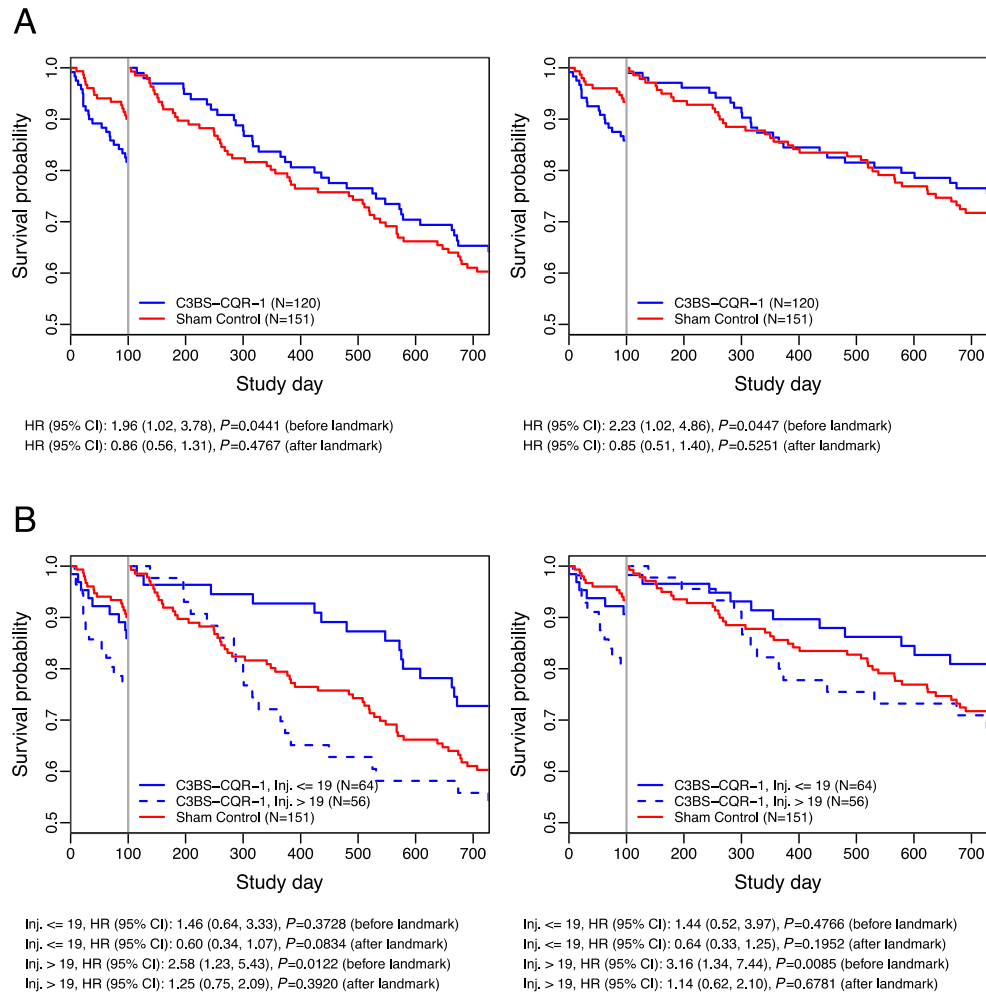HR (95% CI): 0.76 (0.32, 1.79), *P*=0.5313 (after landmark)

or heart failure hospitalization between cell-treated and sham-controlled groups crossed at 100 days, with lower risks in the active group after 100 days (*Figure 3*, upper row). Given the prior observation of an inverse relationship between dosing and reduction in LV volume,[14] we explored the impact of treatment intensity dichotomized by median number of injections (*n* = 19). The event-free survival was characterized by an early hazard in patients treated with 20 or more injections compared with patients receiving ≤19 injections (*Figure 3*, bottom row). At follow-up beyond 100 days, patients receiving ≤19 injections showed lower risks compared with controls although differences were not statistically significant. To determine whether injection-related myocardial damage may play a role, we determined the association of peak post-procedural troponin values as well as the change from baseline to peak post-procedure value with numbers of injections. Troponin assessments were similar across the injection range (Supporting Information, *Figure S1*).

Landmark analyses in population with advanced left ventricular enlargement

In patients with baseline LVEDV of 200–370 mL, the risks of CV hospitalizations and the composite of CV hospitalization and death were lower in patients treated with cell therapy (*Figure 4A*). In this subgroup of patients, risks in the active group appeared to be further reduced after 100 days of follow-up (*Figure 4B*). In patients with LVEDV of 200–370 mL, post-landmark risks of all-cause death or CV hospitalization and CV death or heart failure hospitalization appeared lower in those who received ≤19 injections compared with controls (*Figure 4C*; HR 0.38, 95% CI 0.16–0.91, *P* = 0.031 and HR 0.28, 95% CI 0.09–0.94, *P* = 0.040, respectively).

Corroborating the landmark analysis, post hoc analysis of the primary composite endpoint in this cell-treated patient subgroup demonstrated benefit compared with controls (Supporting Information, *Figure S2*; M–W estimator at 52 weeks 0.71, 95% CI 0.60–0.83, *P* < 0.001). The suggestion

Figure 3 Landmark analyses of modifying effect of number of injections in the entire study population. Kaplan–Meier estimates of death or cardiovascular hospitalization (left panels) and cardiovascular death or heart failure hospitalization (right panels) through Week 104, in patients treated as randomized (treated set) for (A) landmark analysis by treatment group and (B) landmark analysis by median number of injections. CI, confidence interval; HR, hazard ratio.

A

HR (95% CI): 1.96 (1.02, 3.78), P=0.0441 (before landmark)
HR (95% CI): 0.86 (0.56, 1.31), P=0.4767 (after landmark)

HR (95% CI): 2.23 (1.02, 4.86), P=0.0447 (before landmark)
HR (95% CI): 0.85 (0.51, 1.40), P=0.5251 (after landmark)

B

Inj. <= 19, HR (95% CI): 1.46 (0.64, 3.33), P=0.3728 (before landmark)
Inj. <= 19, HR (95% CI): 0.60 (0.34, 1.07), P=0.0834 (after landmark)
Inj. > 19, HR (95% CI): 2.58 (1.23, 5.43), P=0.0122 (before landmark)
Inj. > 19, HR (95% CI): 1.25 (0.75, 2.09), P=0.3920 (after landmark)

Inj. <= 19, HR (95% CI): 1.44 (0.52, 3.97), P=0.4766 (before landmark)
Inj. <= 19, HR (95% CI): 0.64 (0.33, 1.25), P=0.1952 (after landmark)
Inj. > 19, HR (95% CI): 3.16 (1.34, 7.44), P=0.0085 (before landmark)
Inj. > 19, HR (95% CI): 1.14 (0.62, 2.10), P=0.6781 (after landmark)

of an overall benefit in this subgroup of patients was consistent across all components of the composite endpoint at 52 weeks (Supporting Information, *Table S1*). Furthermore, these results were in line with the suggested overall risk reduction of all-cause death or CV hospitalization and CV death or heart failure hospitalization in patients with baseline LVEDV of 200–370 mL who received ≤19 injections compared with controls (HR 0.46, 95% CI 0.23–0.94, P = 0.034 and HR 0.28, 95% CI 0.10–0.79, P = 0.016, respectively; *Figure 5*).

Safety

Cumulative rates of safety events through 52 and 104 weeks in all patients according to treatment received (safety set) are shown in *Table 1*. The median follow-up was 733 days (104.7 weeks). No significant differences in rates of

all-cause mortality at either 52 or 104 weeks post-procedure were observed between patients receiving cell therapy compared with sham control (log-rank test P = 0.96 and 0.39, respectively). Over 2 years, adjudicated causes of death were similar in the two arms, although notably CV death occurred in 36 (21.7%) sham-treated patients vs. 21 (17.6%) cell-treated patients (log-rank test P = 0.48). Incidence of death due to heart failure or sudden cardiac death in the control group vs. the active cell-treated group was 11.4% vs. 8.6% and 7.7% vs. 4.7%, respectively (not significant). Rates of adjudicated, non-fatal safety endpoints including MI, stroke, and aborted sudden death were similar between groups (log-rank test P = 0.72, 0.93, and 0.37, respectively, *Table 1*). The incidence of serious adverse events reported by blinded, assessor investigators through 2 years was also similar between the groups (*Table 1*). The proportion of patients hospitalized one or more times through

Figure 4 Landmark analyses with modifying effect of baseline left ventricular volumes and number of injections. Kaplan–Meier estimates of death or cardiovascular hospitalization (left panels) and cardiovascular death or heart failure hospitalization (right panels) through Week 104, in patients with left ventricular end-diastolic volume of 200–370 mL and treated as randomized for (A) overall analysis by treatment group, (B) landmark analysis by treatment group, and (C) landmark analysis by median number of injections. CI, confidence interval; HR, hazard ratio.
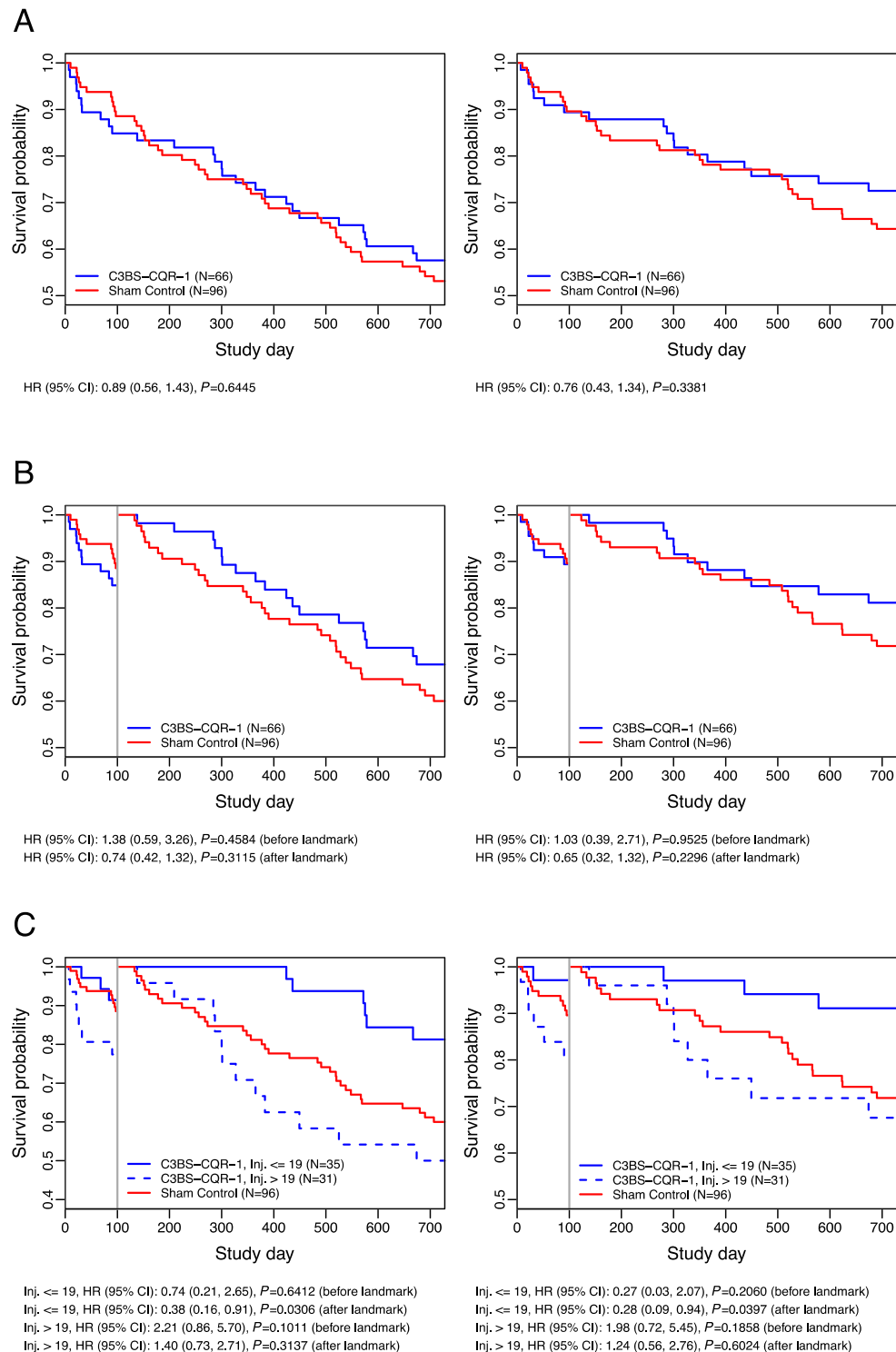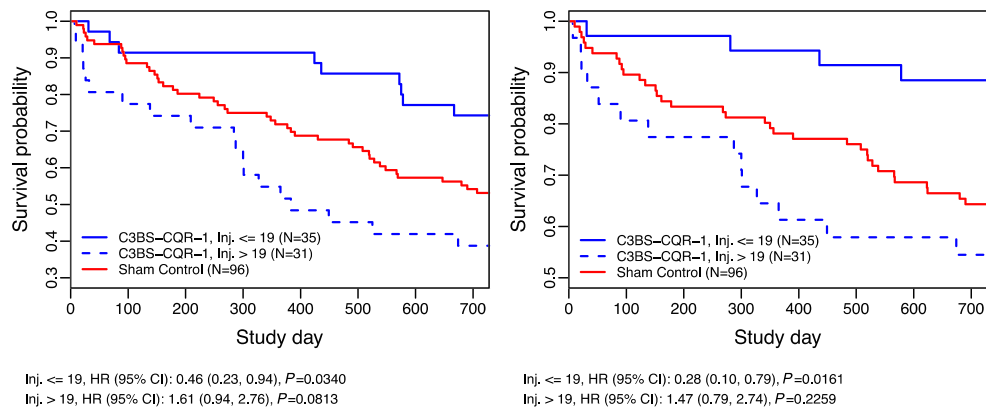
Figure 5 Exploratory analyses of modifying effect of number of injections in subsets of patients with target left ventricular enlargement through 2 years of follow-up. Kaplan–Meier estimates of death or cardiovascular hospitalization (left panel) and cardiovascular death or heart failure hospitalization (right panel) through Week 104, in patients with left ventricular end-diastolic volume of 200–370 mL by median number of injections. CI, confidence interval; HR, hazard ratio.

Inj. <= 19, HR (95% CI): 0.46 (0.23, 0.94), P=0.0340
Inj. > 19, HR (95% CI): 1.61 (0.94, 2.76), P=0.0813

Inj. <= 19, HR (95% CI): 0.28 (0.10, 0.79), P=0.0161
Inj. > 19, HR (95% CI): 1.47 (0.79, 2.74), P=0.2259

104 weeks was also similar between groups (45.2% in control vs. 43.0% in active), with 39.7% and 40.8% admitted for a CV reason. The incidence of adjudicated heart failure hospitalizations was 25.7% in control-treated patients and 29.8% in active-treated patients (log-rank test $P = 0.47$).

Discussion

The durability of clinical outcomes in regenerative heart failure trials remains uncertain.[17] Different approaches to ensure long-term efficacy are thus considered, albeit with undefined success including repeated dosing or enhanced cell retention strategies.[18–20] Here, we leverage the largest regenerative clinical trial in ischaemic heart failure to date to assess outcome up to 2 years following a single-dose administration of lineage-primed cardiopoietic stem cells delivered with a retention-enhanced endomyocardial catheter.

At 1 year of follow-up, the composite efficacy endpoint remained neutral, confirming the previously reported 9 months of experience.[13] A suggestion of potential benefit identified by post hoc analyses in a subset of patients with advanced LV enlargement (baseline LVEDV between 200 and 370 mL) was consistent with the prior experience at 9 months.[13] Extending the clinical follow-up to 2 years indicated favourable clinical outcome in this population at risk. Importantly, the clinical benefit of cardiopoietic cell therapy was achieved upon a background of optimal standard of care suggesting an adjunct value of biotherapy in the management of advanced heart failure. These post hoc analyses suggest that targeted patient selection using disease severity markers should be considered in the design of future clinical trials assessing cell therapy in patients with heart failure. The experience obtained herein is consistent with the recent validation

of baseline LV enlargement as a modifier of therapeutic response in experimental cell therapy.[21] These findings are in line with known relationship between LV volumes and outcomes in heart failure and therapeutic response to traditional medical and device-based interventions in heart failure[22–24] with therapeutic effect being absent once heart failure progressed beyond the point of potentially meaningful clinical impact. Notwithstanding, the experience obtained herein should be considered as hypothesis generating with need for independent validation in prospectively designed trials incorporating target patient selection using the LV volume range as heart failure severity criterion.

In line with a modifying effect of treatment intensity dichotomized by median number of 19 injections on LV volume reduction previously signalled through a 1 year echocardiographic follow-up,[14] the current 2 years of analyses indicated that avoiding excessive treatment intensity was safe short term and may yield long-term clinical benefit with improved survival and reduced cardiac morbidity within the responsive degree of baseline LV enlargement. Absence of a traditional dose-dependent relationship has been previously noted[25–27] and suggests a ceiling effect in particular when utilizing a delivery device with enhanced cell capability. A plausible contributor is myocardial tissue saturation due to the optimized needle–myocardial relationship that favours cell retention in the injected muscle area.[11,15] Importantly, no association with the extent of myocardial injury, as assessed from either peak or changes in cardiac troponin levels,[14] was observed. As control patients underwent a sham procedure with no intramyocardial placebo injections and the active group received a pre-allocated cell concentration and injection volume, putative effects of injection volumes and/or numbers of injections remain to be determined. Accordingly, there is a need to titrate the proper posology in the context of a dedicated delivery system and patient risk profile.

Table 1 Safety events through 52 and 104 weeks in patients as treated (safety set)

	Sham control (N = 170)		C3BS-CQR-1 (N = 120)	
	Week 52 n (KM%)	Week 104 n (KM%)	Week 52 n (KM%)	Week 104 n (KM%)
Total deaths	21 (12.4)	45 (26.5)	15 (12.5)	26 (21.7)
Cardiovascular cause	19 (11.3)	36 (21.7)	15 (12.5)	21 (17.6)
Heart failure/ cardiogenic shock	11 (6.7)	18 (11.4)	8 (6.8)	10 (8.6)
Sudden cardiac death	6 (3.7)	12 (7.7)	2 (1.8)	5 (4.7)
Acute MI	0	0	1 (0.9)	1 (0.9)
Stroke	1 (0.7)	1 (0.7)	1 (0.9)	1 (0.9)
Rhythm disturbances	0	1 (0.7)	1 (0.9)	1 (0.9)
Other cardiovascular cause	0	0	1 (0.8)	1 (0.8)
Undetermined cause	1 (0.6)	4 (2.8)	1 (0.9)	2 (1.9)
Non-cardiovascular cause	2 (1.2)	9 (6.1)	0	5 (5.0)
Infection	2 (1.2)	6 (4.1)	0	1 (1.0)
Pulmonary	0	0	0	1 (1.0)
Renal	0	0	0	1 (1.0)
Haemorrhage, not intracranial	0	1 (0.7)	0	0
Other non-cardiovascular	0	0	0	1 (1.1)
Malignant cause	0	2 (1.4)	0	1 (1.0)
Non-fatal events				
Cardiac transplantation	0	1 (0.8)	1 (0.9)	1 (0.9)
Myocardial infarction	1 (0.6)	2 (1.3)	2 (1.8)	2 (1.8)
During hospitalization for study procedure	0	0	0	0
After hospitalization for study procedure	1 (0.6)	2 (1.3)	2 (1.8)	2 (1.8)
Stroke	3 (1.9)	4 (2.6)	3 (2.6)	3 (2.6)
During hospitalization for study procedure	0	0	1 (0.8)	1 (0.8)
After hospitalization for study procedure	3 (1.9)	4 (2.6)	2 (1.8)	2 (1.8)
Aborted sudden death	5 (3.0)	6 (3.8)	3 (2.7)	7 (6.8)
During hospitalization for study procedure	0	0	0	0
After hospitalization for study procedure	5 (3.0)	6 (3.8)	3 (2.7)	7 (6.8)
Aborted sudden death or sudden cardiac death	10 (6.1)	17 (10.8)	5 (4.5)	12 (11.3)
Adverse events reported by evaluator investigators (blinded)				
Any AE	100 (58.8)	123 (72.4)	74 (62.6)	83 (70.3)
AE related to cardiopoietic cells or sham as reported by investigator	2 (1.2)	3 (1.9)	6 (5.2)	6 (5.2)
AE related to the catheter as reported by investigator	2 (1.2)	3 (1.9)	4 (3.4)	4 (3.4)
Any serious AE	72 (42.4)	99 (58.2)	52 (44.0)	68 (57.6)
Serious AE with fatal outcome	22 (12.9)	45 (26.5)	13 (11.0)	24 (20.3)
Hospitalizations				
Any cause	51 (30.3)	75 (45.2)	36 (30.5)	50 (43.0)
Cardiovascular	46 (27.6)	65 (39.7)	32 (27.2)	47 (40.8)
Heart failure (adjudicated)	32 (19.3)	42 (25.7)	24 (20.5)	34 (29.8)

AE, adverse event; MI, myocardial infarction.
Data are expressed as absolute number of events and Kaplan–Meier estimates (%).

With regard to biological mechanisms of action, *in vivo* tracking and post-mortem analysis in large animal translational studies document limited long-term intramyocardial cell integration, yet significant induction of neo-angiogenesis and recruitment of endogenous progenitors to the infarct border zone as underlying mechanisms of improved LV function, infarct size reduction, and remodelling in the context of cardiopoietic stem cell therapy.[9] This is consistent with the observed reduction in LV volumes 1 year after therapy in the human setting.[14] Moreover, proteomics analysis has resolved nearly 4000 proteins constituting the cardiac proteome, with 450 proteins altered by chronic infarction—a number reduced to 283 by cardiopoietic cell treatment that non-stochastically remediated 85% of disease-affected protein clusters.[28] Systems interrogation suggested vasculogenesis, cardiac development, organ regeneration, and differentiation as integral in defining the molecular outcome underlying a cardiopoietic stem cell intervention-induced transition of infarcted hearts from a cardiomyopathic trajectory towards pre-disease likely through paracrine mode of action.[28]

Several limitations need to be considered in the present study. First, administering a lower number of injections was a decision made on clinical grounds by the operators. Control patients did not undergo placebo injection. In principle, this may confound data interpretation and association with improved outcomes requires formal validation and is hypothesis generating. One might expect that injections would be curtailed in patients intolerant of the procedure or demonstrating other higher risk features, thereby portending a poorer outcome that was however not observed. Patients receiving various numbers of injections had similar baseline characteristics suggesting similar baseline risks.[14] Second, the trial was not powered to detect differences in clinical outcomes. The identification of responders was based on established post hoc analyses,[13] and accordingly, such analyses of clinical outcomes should be considered exploratory and hypothesis generating. Nonetheless, the consistency in outcomes across the longitudinal experience and the continued clinical benefit driven by the accrual of relevant endpoints through the 104 weeks of follow-up warrants additional investigation and validation.

In conclusion, the present longitudinal study extends the neutral readouts reported following intramyocardial injection of cardiopoietic stem cells in an untargeted ischaemic heart failure population. Concomitantly, long-term clinical experience with post hoc analyses suggests a potential for sustained benefit of cardiopoietic cell therapy on clinically relevant endpoints in a target population with a baseline LVEDV between 200 and 370 mL, as long as fewer than 19 injections are employed. Given also the consistency with previous findings on remodelling outcomes,[14] further clinical validation is warranted. In this regard, the Food and Drug Administration granted a 'Fast Track' designation to cardiopoietic stem cell therapy for reduction in mortality, hospitalization, and

improvement in quality of life for patients with chronic heart failure secondary to ischaemic cardiomyopathy with baseline LVEDV between 200 and 370 mL.[29] Thus, clinical experience with cardiopoietic cell therapy invites careful further assessment to delineate potentially responsive patients with advanced ischaemic heart failure using refined selection criteria and relevant treatment approaches. Optimization of the design and execution of clinical protocols for cell-based therapy is a collective prerogative in CV clinical development efforts.[30]

Conflict of interest

J.B. and W.W. have been members of an institution that co-founded Cardio3 BioSciences (now Celyad). J.B. reports that all consultancy/speakers fees and research contracts are directed to Cardiac Research Institute, Aalst, Belgium. A. T. and A.B. report that they are listed as co-inventors on patents US 20080019944 and US 20120100533. They are supported by National Institutes of Health (HL134664), Marriott Family Foundation, Michael S and Mary Sue Shannon Family, Russ and Kathy VanCleve Foundation, and Mayo Clinic Center for Regenerative Medicine. W.W. reports institutional research grants from several device companies (including Biotronik, MiCell, MicroPort, and Terumo); speakers fees from Abbott Vascular, Biotronik, and MicroPort; and co-founder of Argonauts Partners, an innovation facilitator. B.D. reports grants from Celyad, Amgen Inc., Cirius Therapeutics Inc., Laguna Pharmaceuticals, Novartis Pharmaceutical Corp, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, and Ventrix; and personal fees from Novartis Pharma AG. G.C. reports grants from Celyad, Amgen Inc., Cirius Therapeutics Inc., Laguna Pharmaceuticals, Novartis Pharmaceutical Corp, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, and Ventrix; and personal fees from Novartis Pharma AG. S.S. reports grants from Celyad, Amgen Inc., Cirius Therapeutics Inc., Laguna Pharmaceuticals, Novartis Pharmaceutical Corp, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, and Ventrix. W.S. is a Biotechnology Consultant, previously CMO at Celyad. All other authors have no disclosures.

Supporting information

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

Figure S1. Cardiac troponin changes after interventional cell therapy. Geometric means of high-sensitivity troponin peak (left panel) and changes (right panel) with corresponding 95% confidence intervals in relation to number of injections in patients treated as randomized. CI: confidence interval.

Figure S2. Exploratory analyses of modifying effect of baseline LV enlargement and number of injections on primary composite endpoint at one year. The Mann–Whitney estimator, or the probability that the treatment group had a better outcome on the composite primary endpoint, and corresponding 95% confidence interval at 52 weeks in overall patient population, in the subgroup of patients with LVEDV 200–370 mL, in all patients with ≤ 19 injections and in subgroup of patients with LVEDV 200–370 mL and with ≤ 19 injections. The value >0.5 of Mann–Whitney estimator favors the active treatment. The vertical reference line at 0.61 represents the treatment effect at week 39 assumed when designing the study. CI: confidence interval; LVEDV: left ventricle end-diastolic volume.

Table S1. Composite endpoint components at 52 weeks in subpopulation with LV EDV 200–370 mL with <19 injections compared to sham with similar LV enlargement. *Mortality in efficacy analysis defined as death, LVAD or urgent cardiac transplant.

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