Intracoronary Autologous Cardiac Progenitor Cell Transfer in Patients with Hypoplastic Left Heart Syndrome (TICAP): A Prospective Phase 1 Controlled Trial

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Abstract

Rationale: Hypoplastic left heart syndrome (HLHS) remains a lethal congenital cardiac defect. Recent studies have suggested that intracoronary administration of autologous cardiosphere-derived cells (CDCs) may improve ventricular function.

Objectives: The aim of this study was to test whether intracoronary delivery of CDCs is feasible and safe in patients with HLHS.

Methods and Results: Between January 5, 2011, and January 16, 2012, 14 patients (1.8 ± 1.5 years) were prospectively assigned to receive intracoronary infusion of autologous CDCs 33.4 ± 8.1 days after staged procedures (n=7), followed by 7 controls with standard palliation alone. The primary endpoint was to assess the safety and the secondary endpoint included the preliminary efficacy to verify the right ventricular ejection fraction (RVEF) improvements between baseline and 3 months. Manufacturing and intracoronary delivery of CDCs were feasible and no serious adverse events were reported within the 18-month follow-up. Patients treated with CDCs showed RVEF improvement from baseline to 3-month follow-up (46.9 ± 4.6% to 52.1 ± 2.4%, P=0.008). Compared with controls at 18 months, cMRI analysis of CDC-treated patients showed a higher RVEF (31.5 ± 6.8% vs. 40.4 ± 7.6%, P=0.049), improved somatic growth (P=0.0005), reduced heart failure status (P=0.003), and lower incidence of coil occlusion for collaterals (P=0.007).

Conclusions: Intracoronary infusion of autologous CDCs appears to be feasible and safe in children with HLHS after staged surgery. Large phase 2 trials are warranted to examine the potential effects of cardiac function improvements and the long-term benefits of clinical outcomes.

Clinical Trial Registration: Clinicaltrials.gov identifier: NCT01273857.

Abbreviations

HLHS = hypoplastic left heart syndrome

CDCs = cardiosphere-derived cells

RVEF = right ventricular ejection fraction

EDV = end-diastolic volume

ESV = end-systolic volume

Ea = effective arterial elastance

Ees = end-systolic elastance

cMRI = cardiac magnetic resonance imaging

UCG = ultrasonic echocardiography

SPECT = single photon emission computed tomography

BSA = body surface area

Introduction

Hypoplastic left heart syndrome (HLHS), one of the single ventricle lesions, is characterized by hypoplasia of the left ventricle, the aorta, and related valvular components with systemic flow dependent on a patent ductus arteriosus. Infants with this syndrome are at risk of death and require immediate surgery within a few days of birth.[1] Primary neonatal heart transplantation is an option for treatment; however, the morbidity and mortality associated with transplantation is not trivial and the shortage of donors among children has become a critical issue owing to the increase of prenatal diagnosis.[2] The alternative may be staged surgical palliations, including Norwood, Glenn, and Fontan procedures. Although recent advances in the surgical management of HLHS have dramatically changed the early clinical outcome,[3] which was previously lethal, initial right ventricular (RV) dysfunction has shown to be associated with intermediate or late mortality even after successful reconstructions.[4, 5] Additional treatment may be required to compromise the RV-dependent systemic circulation in long-term.

Experimental studies have suggested that the decline of cardiomyocyte replication might be associated with the absolute loss of intrinsic progenitor cells or reduced potential of preexisting mature myocyte proliferation during heart development.[6, 7] In this regard, various types of cytokine or specified molecular-targeted therapy have been shown to promote mature cardiomyocyte proliferation by preclinical evidence;[8, 9] however, we propose here that endogenous cardiac progenitor cells in children, which are more abundant, self-renewing, and multipotent than those found in adults in order to ensure the completion of physiological growth in response to normal demand during cardiac development, might be more clinically applicable and safe to treat patients with heart failure as a therapeutic strategy.[10-12]

An initial clinical trial has shown that intracoronary transfer of CDCs after myocardial infarction could restore substantial myocardial thickening rather than global function through the mechanisms dependent on a reduction in scar size that resulted in increased viable cardiac muscles.[13] However, specific stem/progenitor cells have not been established yet as a standard therapeutic strategy to treat severe heart failure in children, and prospective investigations have also not been reported to verify the clinical impact of the intracoronary administration of autologous CDCs to treat patients with fatal cardiac defects.[14] Our aim here was to investigate prospectively the feasibility, safety, and efficacy of transcoronary infusion of patient-derived CDCs in children with HLHS. To our knowledge, this is the first phase 1 controlled trial to deliver manufactured CDCs into children following standardized staged shunt procedures for critical patients with HLHS.

Methods

Study Design

Between January 5, 2011, and January 16, 2012, we performed a non-randomized, prospective controlled exploratory study including 7 patients constitutively assigned to receive intracoronary infusion of CDCs 4 to 5 weeks after surgical palliations followed by 7 patients allocated to a control group with standard care. Inclusion criteria consisted of a diagnosis of single ventricle physiology with a plan for stage 2 or 3 surgical reconstructions within a month after initial screening by echocardiography. Exclusion criteria were cardiogenic shock, intractable arrhythmias, repeated infections, advanced renal or hepatic dysfunction, manifested cancer diseases, and inability to complete the protocol treatment and examination. The detailed study protocols are described in the Supplemental Material.

To limit problems associated with the variable degrees of complex pathophysiology in patients with single ventricle physiology, we prospectively enrolled children under the age of 6 years old who had been diagnosed with HLHS at initial screening and scheduled for stage 2 or 3 cardiac reconstructions. Patients undergoing the first palliative stage, including the Norwood procedure with a modified Blalock-Taussig (m-BT) shunt or right ventricle-pulmonary artery (RV-PA) shunt, or bilateral PA banding within the first month after birth were excluded owing to the associated high risk. Details of the patients' baseline information are provided in Online Tables I and II.

This study protocol of the Transcoronary Infusion of CArdiac Progenitor Cells in Patients with Single Ventricle Physiology (TICAP) trial was approved in December 2010 by the Ethics Committee of Okayama University and followed the Guidelines on Clinical Research Using Human Stem Cells issued by the Ministry of Health, Labour and Welfare, Japan. The study was performed in accordance with the Declaration of Helsinki with written consent from all parents of eligible patients.

Study Endpoints

The feasibility evaluation for procedural complications was determined by distal coronary embolization, coronary artery injury, and sustained ventricular arrhythmia associated with CDC infusion. The primary safety endpoints at 3 months were cardiac death due to ventricular fibrillation, ventricular tachycardia, and myocardial infarction after CDC infusion. The secondary endpoints were the incidence of hospitalization for heart failure, ventricular arrhythmia, general infection, and renal and hepatic dysfunction by CDC treatment. As a preliminary result of the efficacy endpoints, cardiac function at 3 months after CDC transfer and in controls at corresponding intervals was also evaluated by echocardiography, RV angiogram, and cMRI.

Cell Validation

Immunofluorescence of grown CDCs revealed that these cells expressed SIRPA (signal-regulatory protein α: a cell surface marker to identify cardiac lineage-committed cells) but lacked discoidin domain receptor 2 (DDR2) expression, a collagen receptor to recognize cardiac fibroblasts (Figure 1A).[15, 16] Flow cytometric analysis confirmed these observations and showed that CDCs were negative for CD31, CD45, and the cardiac structural gene tropomyosin (Figure 1B). Individual patient-derived CDCs were validated precisely in accordance with the protocol. As manufacturing criteria, more than 60% of the CDCs expressed SIRPA and mesenchymal stem cell markers, such as CD90, CD105, and vimentin, but fewer than 1% could be detected using CD31, CD45, and DDR2 (Figures 1C). Compared with cardiac fibroblasts, patient-derived CDCs expressed typical cardiac transcription factors such as GATA4, Mef2c, Tbx5, Hand2, Myocardin, Mesp1, and Nkx2.5, as well as a vascular endothelial progenitor marker, Flk1. Unlike cardiac fibroblasts expressing elastin, CDCs had greater expression of typical cardiac ion channel genes and their regulatory transporters include ryanodine receptor 2 (Ryr2), sarcoplasmic reticulum Ca^+-ATPase 2 (SERCA2), and inositol trisphosphate (IP3), but remained undifferentiated by the lack of mature cardiac structural proteins such as cardiac troponin-T, α-myosin heavy chain (MHC), myosin light chain (MLC)-2v, and natriuretic peptide A (Figure 1D).

Cell Infusion

For patients allocated to receive CDCs, expanded CDCs were harvested, calculated, and prepared as $3.0x10^5$ cells per kilogram of body weight on the day of infusion, based on previous preclinical and clinical studies.[13, 17] For patients assigned as controls, we did not perform the placebo injection during cardiac catheterization one month after surgical shunt procedures owing to ethical considerations. Validated CDCs were diluted into 3 mL of autologous serum containing growth medium supplemented with 100 units of heparin/mL. Heart catheterization was performed under general anesthesia 4 to 5 weeks after staged shunt procedures. The femoral artery was punctured and a 5 French sheath was placed. A 2.8 French temporary occlusion balloon catheter Iiguman type C (Fuji Systems Corporation) was selectively advanced into the coronary arteries through 0.012'' guide wire with the backup support of a 5 French Launcher guiding catheter. For the CDC transfer, the balloon was inflated at a low pressure to block the blood flow completely for 2 min while the progenitor cell suspensions were selectively infused into each coronary artery (1~1.5 ml by one infusion per coronary artery) through the distal site of the occluding balloon (Online Figure I).[18] No patients had single coronary distribution. CDCs could be selectively infused into three coronary arteries in 6 out of

7 patients. One patient had severely underdeveloped left circumflex branch and was treated by two injections. To avoid cell transfer-induced arrhythmia, amiodarone (10 μg/kg/min) was intravenously infused 30 min prior to the coronary injection during the catheterization.[17]

Cardiac Function Analysis

Two-dimensional and Doppler echocardiograms were produced using an IE33 transducer (Philips Medical Systems). Right ventricular ejection fraction (RVEF), end-systolic volume (ESV), and end-diastolic volume (EDV) were calculated using the monoplane ellipsoid approximation method from a transverse apical 4-chamber view area at the level of the tricuspid annulus and the distance from the tricuspid valve center to the apex.[19] The degree of tricuspid regurgitation was assessed by the width of regurgitant jet vena contracta from the orifice and quantitatively classified. Tricuspid valve dimension was measured by the maximal annulus diameter in the apical 4-chamber view and corrected by z-value nomograms based on body surface area.[20]

Quantitative analysis of paired RVEF was carried out, and ESV, EDV, stroke volume, and cardiac output were calculated from recorded cine images by tracing endocardial contours in end-diastolic and end-systolic phases.[21] Data were analyzed using elk C View Ver. 1.7 (ELK Corporation). The approximations of Ees (end-systolic elastance) and Ea (effective arterial elastance) were calculated by pressure study and volume measurement, as previously reported.[22]

cMRI scans were performed on a Philips 1.5 Tesla Achieva Scanner (Philips Healthcare, Netherlands) under general anesthesia. The phased array coil was selected. Four-electrode vector electrocardiography was used for cardiac triggering. Short-axis cine images through the heart were obtained to quantify single ventricle volume and function using a steady-state-free precession sequence (parameters: repetition time, 3.2 msec; echo time, 1.6 msec; acceleration factor, 2; flip angle, 60 degrees; field of view, 200 mm; matrix, 128×128; and slice thickness, 5 mm).[23] The basal short-axis slice was positioned beyond the level of the tricuspid valve, and the entire RV was imaged from the base toward the apex in contiguous slices. The RVESV, RVEDV, stroke volume, and RVEF were calculated from a stack of short-axis cine images using the disc summation method after tracing endocardial contours in end-diastole and end-systole. RVEF was calculated as RV stroke volume, measured by EDV-ESV, which was divided by EDV as previously described. Values of EDV, ESV, and wall mass were indexed to BSA[1.3]. Each study was analyzed and reviewed by experienced pediatric cardiologists and radiologists specialized in echocardiography, angiography, and cMRI who were blinded to the group assignment during the assessment.

Statistics

Data are reported as means ± SD or number (%). A sample size of 7 patients per group was designed to define the feasibility and preliminary safety of CDC infusion without power consideration.[24] Efficacy analyses were descriptive and exploratory with no statistical hypothesis testing was planned in this pilot trial. Baseline characteristics, safety outcome, and adverse events between groups were compared using Fisher's exact test to determine the categorical variables presented by the number of observations. For continuous measures, normality of data was tested using the Shapiro-Wilk test. Measurement of cardiac enzymes and tumor markers in CDC-treated patients during follow-up was assessed using one-way analysis of variance (ANOVA) with repeated measures. No adjustments for multiple comparisons were made in safety endpoints evaluation.[25] Differences in mRNA expression, baseline hemodynamics, the absolute changes in tricuspid annulus diameter and RVEF as well as serum BNP levels and quality of life assessment at 18 months between the control group and the CDC-treated group were analyzed using 2-tailed unpaired Student's t-test if data were distributed normally.[26] When multiple comparisons for cardiac function analysis, somatic growth, and heart failure status assessment were performed within group, ANOVA with repeated measures was conducted and Dunnett post-hoc correction were employed to adjust the type I error due to multiplicity.[27] Significance of the probability value was set at <0.05.[28] As for between-group comparisons in cMRI, 2-way ANOVA was used to analyze the categorical independent variables between groups and the time interaction term within group. Kruskal-Wallis with Steel post-hoc test was applied for ordinal scale analysis in Ross classification. Log-rank test was used to analyze the distribution of time to the earliest event of coil occlusion for collaterals between CDC-treated and control groups. $P<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS software, version 19 (IBM). JMP, version 11.2 (SAS Institute, Cary, NC) was used for ordinal scale analysis.

Results

Patient Enrollment

A total of 94 patients with single ventricle lesions met the eligibility criteria and 18 patients (19%) diagnosed with HLHS were prospectively enrolled in this study (Figure 2A). The baseline characteristics did not differ between the groups of CDC-treated and control patients, except that premature babies were more prevalent in the control group (Table 1). Of the first 10 consecutive patients allocated to receive CDC infusion into the coronary artery, three patients were excluded for the following reasons: one patient underwent pacemaker implantation during cardiac surgery and was unable to undergo cMRI examination, cell processing failure occurred in one patient by bacterial contamination, and one patient had repeated lung infection after the Fontan procedure. The remaining 8 patients were prospectively assigned as controls, who underwent staged palliations without cell transfer. One patient was excluded due to myocardial ischemia that occurred by ostial stenosis at the right coronary artery after surgery.

Baseline examination was performed by cMRI, echocardiography, and angiography to qualify the patients and there were no significant differences between the groups (Online Table I). Patients assigned to the CDC-treated group received CDC infusion (3.0×10^5 per kilogram of body weight) one month after staged surgical procedures. Paired cMRI, angiogram, echocardiogram, and gated-SPECT analysis were performed to assess the global cardiac function and myocardial perfusion (Figure 2B). In contrast, controls were treated by routine care with no cell infusion while undergoing protocol-based safety and efficacy analysis by cMRI, echocardiography, and angiography.

Evaluation of Feasibility and Safety

Intracoronary infusion of CDCs was successfully achieved in all patients. No serious adverse events were reported during the first week after CDC infusion. Myocardial ischemia and perfusion were monitored by ECG recording, Holter monitoring, and gated SPECT analysis at baseline through 3 to 18 months after CDC infusion and no reports of myocardial ischemia were identified during the follow-up period in 7 patients, except for transient ST segment elevation or minimally reduced systemic blood pressure during balloon inflations (data not shown). One patient had a small amount of creatinine kinase (CK) release, but the level of the MB subunit (CK-MB) was within the normal range. The average CK-MB was 12.3 ± 3.7 IU/L at 24 h and 12.1 ± 2.8 IU/L at 48 h after cell administration and remained unchanged at 1 week (15.1 ± 2.8 IU/L). Serum values of cardiac troponin-I were ranged within normal limit before and after CDC infusion, besides one patient with poor RV function showed abnormal values at baseline through 1 week follow-up (Online Figure II).[29] There were no reports of major adverse cardiac events including death and hospitalization for heart failure or sustained ventricular tachycardia

(Table 2). To avoid unexpected tumor formation after cell infusion, the cytogenetic integrity was confirmed, namely, that every sample of CDCs contained normal chromosomes (data not shown). No patients had tumor formation assessed by echocardiography and cMRI, as well as tumor marker measurements, during the follow-up studies (Online Figure II). Two patients from the CDC-treated group underwent balloon dilation in the shunt conduit or the fenestration site during the observation period, those were comparable with controls, but no patients in the CDC-infusion group required coronary artery intervention and coil embolization of aorto-pulmonary collateral arteries by 18 months of follow-up (Table 2 and Figure 6E).

Efficacy of CDC Infusion

With respect to the secondary endpoint, a total of 7 patients received CDC infusion and 7 patients in the control group had paired echocardiogram examination at baseline, and at 3, 6, 12, and 18 months. Baseline and follow-up measurements for the efficacy endpoints are shown in Figure 3. Compared to baseline, cardiac function in the CDC-treated patients had significantly increased from $46.9 \pm 4.6\%$ to $52.1 \pm 2.4\%$ at 3 months (P=0.008), $53.9 \pm 4.0\%$ at 6 months (P=0.0004), $54.7 \pm 3.3\%$ at 12 months (P=0.0001), and $54.0 \pm 2.8\%$ at 18 months (P=0.0004) of follow-up (Figure 3A). By contrast, in the 7 control patients from 3 months to 18 months of follow-up, no significant improvements were found ($46.7 \pm 4.4\%$ at baseline vs. $47.7 \pm 5.3\%$ at 3 months; $48.3 \pm 4.7\%$ at 6 months; $48.5 \pm 5.4\%$ at 12 months; $48.7 \pm 6.7\%$ at 18 months, P=0.49). These results suggest that CDC infusion may continuously exert its beneficial effects over the following 18 months. Analysis of tricuspid valve dimension is shown in Figure 3B and demonstrated that CDC infusion significantly reduced the annulus diameter over time (2.1 ± 0.5 assessed by z-score at baseline to 1.4 ± 0.4 at 18 months, P=0.04), whereas no changes were observed in the control group (2.0 ± 0.3 at baseline to 2.2 ± 0.8 at 18 months, P=0.57). The absolute changes in tricuspid valve dimension from baseline to 18 months did significantly differ between the groups (Figure 3C).

Additional cardiac function assessments by angiography were performed to verify the functional improvements observed in echocardiographic analysis after CDC infusion (Figure 4A). Global RVEF measured by angiography showed a significant increase from baseline to 3 months of follow-up ($52.7 \pm 9.8\%$ to $58.5 \pm 6.7\%$, P=0.03) and this persisted at 18 months ($60.8 \pm 6.1\%$, P=0.002). Patients receiving CDC infusion had greater changes in RVEF than in the controls at 18 months of follow-up (Figure 4B). This increase in cardiac function in the CDC-treated group was associated with a reduction in the RVESVI (55.6 ± 30.5 mL/BSA$^{1.3}$ at baseline to 37.8 ± 12.2 mL/BSA$^{1.3}$ at 18 months, P=0.02), whereas the RVEDVI in both groups remained unchanged at 18 months compared with that at baseline, Figures 4C and D). On the basis of the cardiac catheterization data, cardiac contractility and mechanical efficiency were

evaluated (Figures 4E-G). Compared with baseline, CDC-treated patients showed a significant increase over time in end-systolic elastance measured by Ees (1.5 ± 1.0 mmHg/mL/m^2 at baseline to 2.1 ± 1.0 mmHg/mL/m^2 at 12 months, $P=0.03$; and 2.2 ± 0.9 mmHg/mL/m^2 at 18 months, $P=0.007$), whereas that in the control group was comparable during the follow-up period. The indicators of afterload addressed by Ea in both groups showed no difference from baseline to 18 months later. These findings indicated an improvement of Ea/Ees (ventriculoarterial coupling) in CDC-treated patients but not in controls compared with that at baseline (1.3 ± 0.5 at baseline to 0.8 ± 0.2 at 12 months, $P=0.006$; and 0.8 ± 0.2 at 18 months, $P=0.006$).

All CDC-treated patients underwent cMRI evaluation to address cardiac function. We found that the salutary effects of CDC transfer on global functional improvements at 12 months were consistent with a marked increase in RVEF ($36.1 \pm 7.5\%$ at baseline to $42.7\% \pm 8.7\%$, $P=0.04$; Figure 5A). Although the functional improvements in RVEF were not significant at 18 months, the progressive reductions in RVEDVI and RVESVI over the follow-up stage were evident in CDC-treated patients compared with the levels at baseline examination (RVEDVI: 139 ± 43.4 mL/BSA$^{1.3}$ at baseline to 112.2 ± 31.4 mL/BSA$^{1.3}$ at 18 months, $P=0.007$; RVESVI: 91.6 ± 37.5 mL/BSA$^{1.3}$ at baseline to 67.9 ± 23.6 mL/BSA$^{1.3}$ at 18 months, $P=0.01$; Figures 5B and C). Notably, right ventricular wall masses corrected by BSA$^{1.3}$ and EDV were significantly decreased at 18 months compared with those at baseline (Figures 5D and E). In addition, these long-term benefits by CDC infusion were confirmed by gated SPECT analysis during 12 months of follow-up ($48.0 \pm 14.0\%$ at baseline to $58.9 \pm 14.3\%$ at 12 months; Figure 5F). In this study, 7 control patients underwent cMRI analysis at baseline and 18 months of follow-up (Figures 5G-I). The baseline measurements by cMRI did not differ between CDC-treated and control subjects, whereas global RVEF at 18 months in the CDC-treated group was greater ($31.5 \pm 6.8\%$ vs. $40.4 \pm 7.6\%$), which was associated with reduced RVESVI at 18 months compared with the controls (103.8 ± 36.5 mL/BSA$^{1.3}$ vs. 67.9 ± 23.6 mL/BSA$^{1.3}$).

Somatic Growth and Functional Status after CDC Infusion

We next investigated somatic growth for enrolled patients having staged palliation with or without CDC infusion. Height and weight values were corrected by z-score for analysis. For patients receiving CDC treatment, there were significant increases in z-score from baseline to 12 and 18 months of follow-up (-3.9 ± 2.7 at baseline to -2.6 ± 1.7 at 12 months, $P=0.01$; -2.0 ± 1.4 at 18 months, $P=0.0005$), whereas no significant changes were observed in controls during the corresponding intervals (Figure 6A). Heart failure status was monitored using the Ross classification and the New York University Pediatric Heart Failure Index (NYUPHFI) score between the groups. As shown in Figure 6B, CDC-treated patients demonstrated greater

functional improvements from baseline to 18-month follow-up than the controls (2.4 ± 0.8 at baseline to 1.4 ± 0.5 at 18 months). When the medical regimen was considered for qualification, NYUPHFI scores in CDC-treated and control patients at baseline did not differ (10.2 ± 4.4 vs. 10.0 ± 2.3); however, the CDC-treated group showed a marked reduction in the severity of heart failure during the stages evaluated (7.0 ± 1.5 at 12 months and 6.1 ± 0.9 at 18 months), whereas no significant improvement was observed in controls (Figure 6C). The reduction of heart failure status in CDC-treated patients was clearly associated with a decrease in BNP levels 18 months after CDC infusion compared with that in controls (26.3 ± 28.5 pg/mL vs. 68.6 ± 42.4 pg/mL; Figure 6D). In contrast to the control group, no patients receiving CDC transfer required unintended coil intervention during the 18-month follow-up (P=0.007, Figure 6E).

Discussion

Mortality associated with stage shunt procedures in children with HLHS remains the highest among the congenital heart diseases. When the degree of hypoplasia is severe, the left ventricle is no longer capable of supporting the systemic circulation, immediately leading to fatal pump failure. Although complex reconstruction palliations in infancy have remarkably improved the critical hemodynamics and could provide patients with compromised single ventricle circulation through passive pulmonary blood flow, late developed RV dysfunction has become one of the issues that may impact on survival during staged palliation or even after Fontan completion in HLHS.[30] Against this background, extensive investigations have been reported in the setting of experimental heart failure models with a variety of stem/progenitor cell or induced pluripotent stem cell transplantations to reverse cardiac dysfunction.[31, 32]

Few clinical studies of autologous stem/progenitor cell delivery have been undertaken to investigate the feasibility, safety, and therapeutic efficacy in children with congenital heart diseases including HLHS. [33, 34] Accumulating case reports have shown that bone marrow-derived cells might have possible beneficial effects in patients with congenital heart disease. These reports provide the clinical evidence that intracoronary administration of bone marrow-derived cells is feasible and safe; in some cases, a definitive improvement was found that was evaluated by the increase in ejection fraction and reduced level of BNP, as well as the decrease in functional heart failure status assessed by the New York Heart Association (NYHA) classification. However, these observations from independent case reports are difficult to interpret because of the lack of control subjects as pilot trials to document conclusively the impact of bone marrow-derived cell infusion on heart failure in children.

We and others have reported that human CDCs might be an alternative cell type to treat patients with ischemic cardiomyopathy.[17, 35] By a series of direct comparative studies with bone marrow-derived mononuclear cells and mesenchymal stem cells, CDCs appear to have the strongest therapeutic potential to alleviate ventricular remodeling after infarction through substantial cardiovascular differentiation and paracrine factor secretion in situ.[36] We report here a proof-of-concept TICAP phase 1 controlled trial that intracoronary infusion of autologous CDCs in patients with HLHS was feasible and safe and the preliminary results showed a marked increase in RVEF compared with control subjects without progenitor cell transfer. Notably, these functional improvements were associated with increase in somatic growth and reduction in heart failure status, fewer incidence of catheter intervention which could not be seen in controls. The significant reductions in right ventricular volume, tricuspid annulus diameter, and wall mass in CDC-treated patients rather than in controls suggest a potential effect of this therapeutic approach.[37] A recent report has also shown that myocardial fibrosis detected by cMRI in single ventricle physiology could be closely associated with cardiac dysfunction with

increased ventricular volume and wall mass after Fontan reconstruction.[38] These findings are consistent with the fact that volume reduction by staged palliation could enhance ventricular contractility as well as mechanical efficiency, as shown by cardiac elastance measurement in this study.[22] Thus, the functional benefits of CDCs isolated from children in this study could be interpreted, at least in part, by previous reports on mouse and human demonstrating that the intrinsic pool of cardiac progenitor cells in human neonatal heart was found to have a higher capability to regenerate cardiomyocytes than that in adult myocardium.[6, 7, 39]

The sample size of this exploratory trial was set as small, based on feasibility and the results of our preclinical trial, which could limit the statistical rigor and power and might not be sufficient to draw the preliminary conclusions related to safety and efficacy.[24] With such small numbers of patients, there could be imbalance in pre-registration covariates, which might need adjustment in the analysis. This study was neither powered for clinical outcomes in the groups of 7 patients each nor designed to test formal hypothesis. Although the comparison-wise error due to multiplicity was adjusted by post-hoc correction in situation in which contrasts are limited to comparisons with baseline between the means of active treatment groups, significance of the probability value should be carefully determined.[28] There was a lack of randomization as well as cMRI analysis during midterm follow-up in controls for longitudinal assessment; thus, the results must be considered with caution.[40] Ethical concerns have limited the detailed assessment in the control group because the children might be more frequently exposed to radiation and general anesthesia as an undesired result. The rarity of specified disease as well as the difficulties in recruitment of a sufficient sample population might be alternative reasons for this study being limited as a non-randomized trial. There is limited scope to adjust for the small number of study patients; hence, the covariates may affect the difference between the dependent variable and other independent variables of primary interest in this trial. However, the continuing improvement found in RVEF at 18 months in the CDC-treated group is noteworthy as an initial result of this proof-of-concept study. In addition, potential cell biological mechanisms for enhancing cardiac function, including cell homing, migration, engraftment, and differentiation in patients after intracoronary infusion, remain unclear in this study. CDC-derived progenitor cell recruitment and paracrine effects to secrete survival signals on transferred myocardium might also be associated with a fundamental increase in functional cardiomyocytes to augment the substantial cardiac output.[30] To address these issues, a prognostic cell-tracking system may be required as new translational medicine for children.

There are several limitations to this study. The shunt types that participants received might differ between the control and CDC-treated groups. Our results showed that the functional improvements after CDC infusion tended to be seen in both patients undergoing stage-2 and -3 palliations, regardless of the patients with Fontan physiology possibly having elevated systemic

venous pressure and lower cardiac output. Recent reports from the Pediatric Heart Network Multicenter Study showing a small or modest increase in RVEF in long-term outcomes after staged procedures suggest a possible contribution of CDCs to RV functional improvement in this trial.[37, 41-43] The oxygen saturation did not differ between the 2 groups at baseline; however, the procedure variance might have affected the cumulative events of venous collateral embolization before the completion of Fontan circulation during long-term observation. Satisfactory results for parents and family, such as in terms of survival and quality of life after intervention, remain to be elucidated. Although our investigation by using a parenting stress test and an infant and toddler quality of life questionnaire could not fully address these questions, and the beneficial effects including in terms of exercise tolerance after treatment might take years to develop in children (Online Figure III).[44]

Our prospective controlled study, the first pediatric phase 1 clinical trial of stem cell therapy for heart disease to our knowledge, suggests that intracoronary infusion of autologous cardiac progenitor cells is a feasible and safe approach to treat children with HLHS. There were no obvious adverse side effects, including acute ischemia, proarrhythmia, systemic infection, and tumor formation at up to 18 months of observation. Importantly, we found that this novel therapeutic strategy showed significant functional improvement in RV from 4 months to 18 months after staged cardiac reconstructions compared with that in patients who received standardized cardiac palliation alone. Our work has shown that the resident cardiac progenitor cells in children may have strong potential to improve hypoxic conditioned myocardium compared with other cell types being reported. Based on the preliminary results of this pilot trial, statistically rational approach should be undertaken to design adequately sized definitive pivotal trials for further investigation in accordance with the guideline from the Cardiovascular Cell Therapy Research Network (CCTRN).[25] Enrolling cases of a wide range of single ventricle lesions in addition to HLHS patients are needed to address the beneficial effects of CDC transfer on clinical outcomes, such as cardiac growth development, incidence of heart failure, and survival, in this complex congenital heart disease.

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Table 1. Baseline characteristics of eligible patients in control and CDC-treated groups

	Control group (n=7)	CDC-treated group (n=7)	*P* value
Age at operation (years)	1.5 ± 1.7	2.1 ± 1.2	0.47
Male sex	5 (71)	4 (57)	0.50
Gestation age <38 wks	2 (29)	0 (0)	0.23
Birth weight (g)	2726 ± 291	2891 ± 262	0.29
Body weight at operation (kg)	6.8 ± 3.8	9.2 ± 3.8	0.27
Anatomic diagnosis			
Hypoplastic left heart syndrome	7 (100)	7 (100)	0.65
Mitral stenosis	3 (43)	2 (29)	0.50
Aortic atresia	4 (57)	5 (71)	0.50
Moderate to severe TR	1 (14)	0 (0)	0.50
Associated anomaly			
Right aortic arch	0	1 (14)	0.50
Bilateral SVC	1 (14)	0 (0)	0.50
Primary shunt type (RV-PA)	6 (86)	5 (71)	0.50
Stage 2 palliation	5 (71)	3 (43)	0.29
Stage 3 palliation	2 (29)	4 (57)	0.29
BNP (pg/ml)	53.8 ± 27.3	53.1 ± 48.6	0.97
Oxygen saturation (%)	84.9 ± 7.9	87.8 ± 9.9	0.69
History of bilateral PA banding	2 (29)	3 (43)	0.50
History of catheter interventions			
Balloon arterial septostomy	2 (29)	1 (14)	0.50
APCA coil occlusion	3 (43)	4 (57)	0.50
Medication profile			
Diuretics	7 (100)	7 (100)	0.65
Digitalis	3 (43)	3 (43)	0.70
ACE inhibitor or ARB	4 (57)	5 (71)	0.50
PDE5 inhibitor	3 (43)	1 (14)	0.28
Endothelin-1 receptor antagonist	2 (29)	2 (29)	0.72
Aspirin	4 (57)	3 (43)	0.50

Abbreviations: n, number; RV, right ventricle; TR, tricuspid valve regurgitation; SVC, superior vena cava; PA, pulmonary artery; BNP, B-type natriuretic peptide; APCA, aorto-pulmonary collateral artery; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; PDE5, phosphodiesterase type 5. Data are expressed as number (%) or mean ± SD.

Table 2. Primary outcomes and adverse events during 18-month follow up

	Control group (n=7)	CDC-treated group (n=7)	*P* value
Death (n)	0	0	
ECMO (n)	0	0	
Cardiopulmonary resuscitation (n)	0	0	
Re-hospitalization for heart failure (n)	2	0	0.23
Myocardial ischemia (n)	0	0	
Ventricular arrhythmia* (n)	0	0	
Cerebral infarction (n)	0	0	
Unplanned CCU admission (n)	1	0	0.50
Unplanned intubation (n)	1	0	0.50
Allergic reaction (n)	N/A	0	
PLE (n)	1	0	0.50
Infection (n)	0	0	
Tumor formation (n)	N/A	0	
Cumulative total adverse events (n)	5	0	0.01
Adverse events per patient (n)	0.71	0	
Unintended catheter intervention (n)			
APCA coil occlusion	5	0	0.01
Balloon dilation of RV-PA shunt	0	1	0.50
Balloon dilation of pulmonary artery	2	1	0.50
Balloon dilation of aortic arch	0	1	0.50
Balloon dilation of fenestration	1	1	0.76

*, Sporadic ventricular extra-systoles were excluded. N/A, not applicable; ECMO, extracorporeal membrane oxygenation; PLE, protein-losing enteropathy; CCU, cardiac care unit

Novelty and Significance

What Is Known?

- Hypoplastic left heart syndrome (HLHS) is one of the severe congenital heart diseases, characterized by underdeveloped left sided heart and related components.
- Three staged shunt procedures are the major treatment modalities but the cardiac function during interstage to long-term follow up may not be optimal.
- Resident cardiosphere-derived cells (CDCs) in infants have been shown to exert greater regenerative potential than those from adult myocardium.

What New Information Does This Article Contribute?

- This first phase 1 clinical trial for pediatric heart disease has shown that intracoronary infusion of autologous CDCs is feasible and safe to treat the children with HLHS 1 month after staged palliations.
- Patient-derived CDCs could improve right ventricular function from 3 through 18 months of follow-up period.
- Autologous CDCs infusion could increase the somatic growth and reduce heart failure status during follow-up stage compared with control patients treated by standard palliation alone.

This study is the first clinical trial to report the feasibility and safety of intracoronary injection of cardiac progenitor cells to treat the critical congenital heart defects such as HLHS in children. The safety concerns were intensively monitored by cardiac enzyme measurement, Holter ECG, cMRI, and single photon emission computed tomography (SPECT) analysis during cell infusion toward 18 months of follow-up. There were no reports of pro-arrhythmic events, disturbed myocardial perfusion, and tumor formation during the observational period. Technical difficulties during catheter delivery were not found even the hypoplastic artic arch was reconstructed by Norwood procedure. This proof-of–concept study has shown that the resident cardiac progenitor cells in children may have a great potential to improve right ventricular dysfunction after staged palliations compared with other cell-types being reported. The improved hemodynamics by CDC infusion may enhance mechanical performance in right ventricle, resulting in the prevention of somatic growth failure and catheter-based collateral intervention required even after successful shunt procedures. This work provides a pivotal rationale to conduct further studies in children with single ventricle lesions who may have poor prognosis even after heart transplantation or completion of the staged surgical palliations in young age.
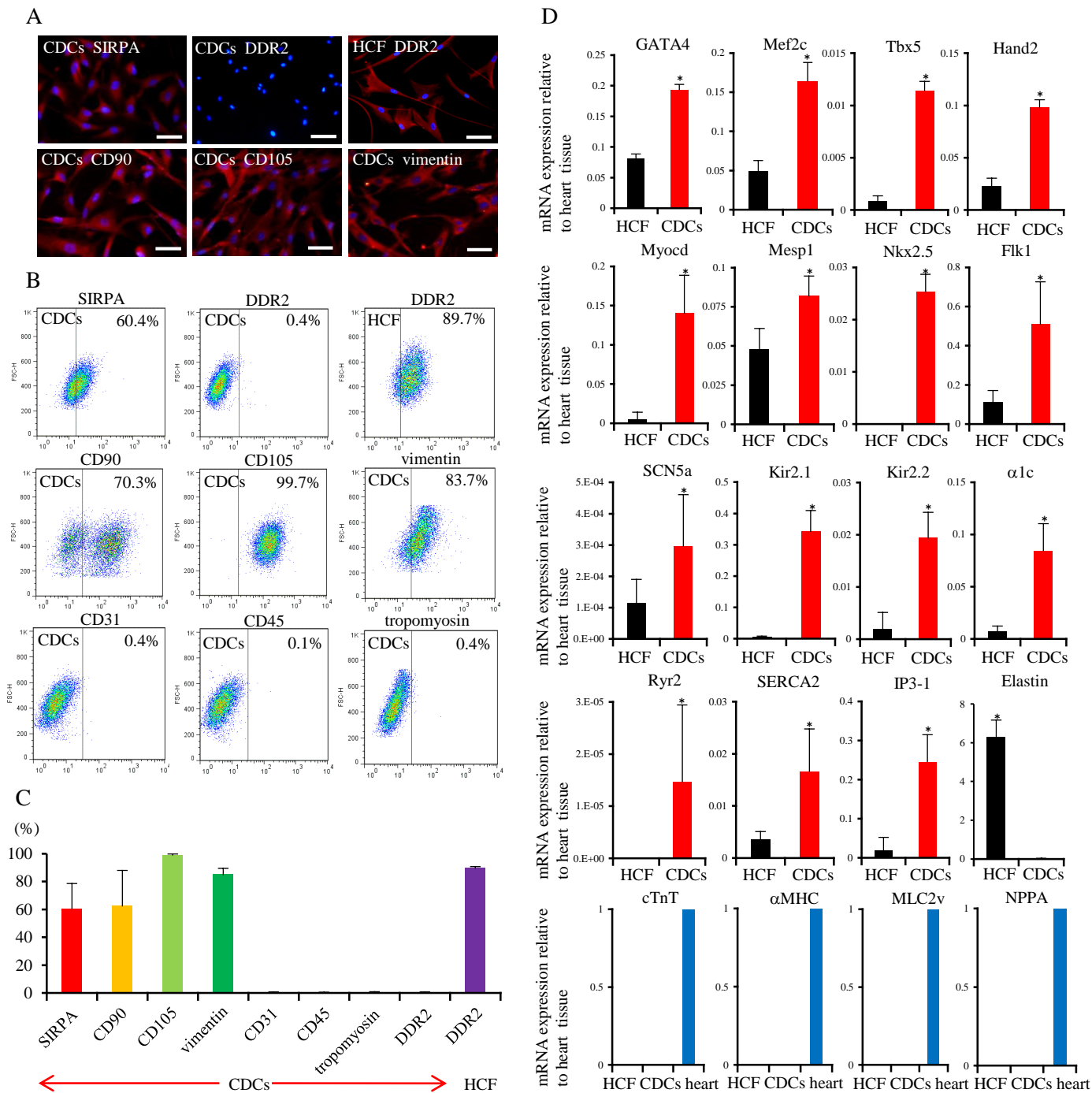
Figure 1. CDC processing and verification.
(A) Cardiospheres were mechanically selected and re-plated onto adherent culture to expand as CDCs. Immunofluorescence by specific antibodies was shown. Patient-derived CDCs expressed typical mesenchymal stem cell markers, CD90, CD105, and vimentin. Human cardiac fibroblasts (HCF) were used as controls for DDR2 staining. Bars, 50 mm. (B) FACS analyses were performed to assess the quality of CDCs as characteristically consistent with cardiac progenitor cells. (C) Summary of FACS data. CDCs expressed SIRPA, but were negative for DDR2 that is present in HCF. (D) Real-time RT-PCR analysis to verify the amplified CDCs before intracoronary infusion. Essential cardiac transcription factors, cardiac ion channel genes, and structural genes are shown. *, $P<0.05$ vs. HCF, analyzed by 2-tailed unpaired Student's t-test.

A
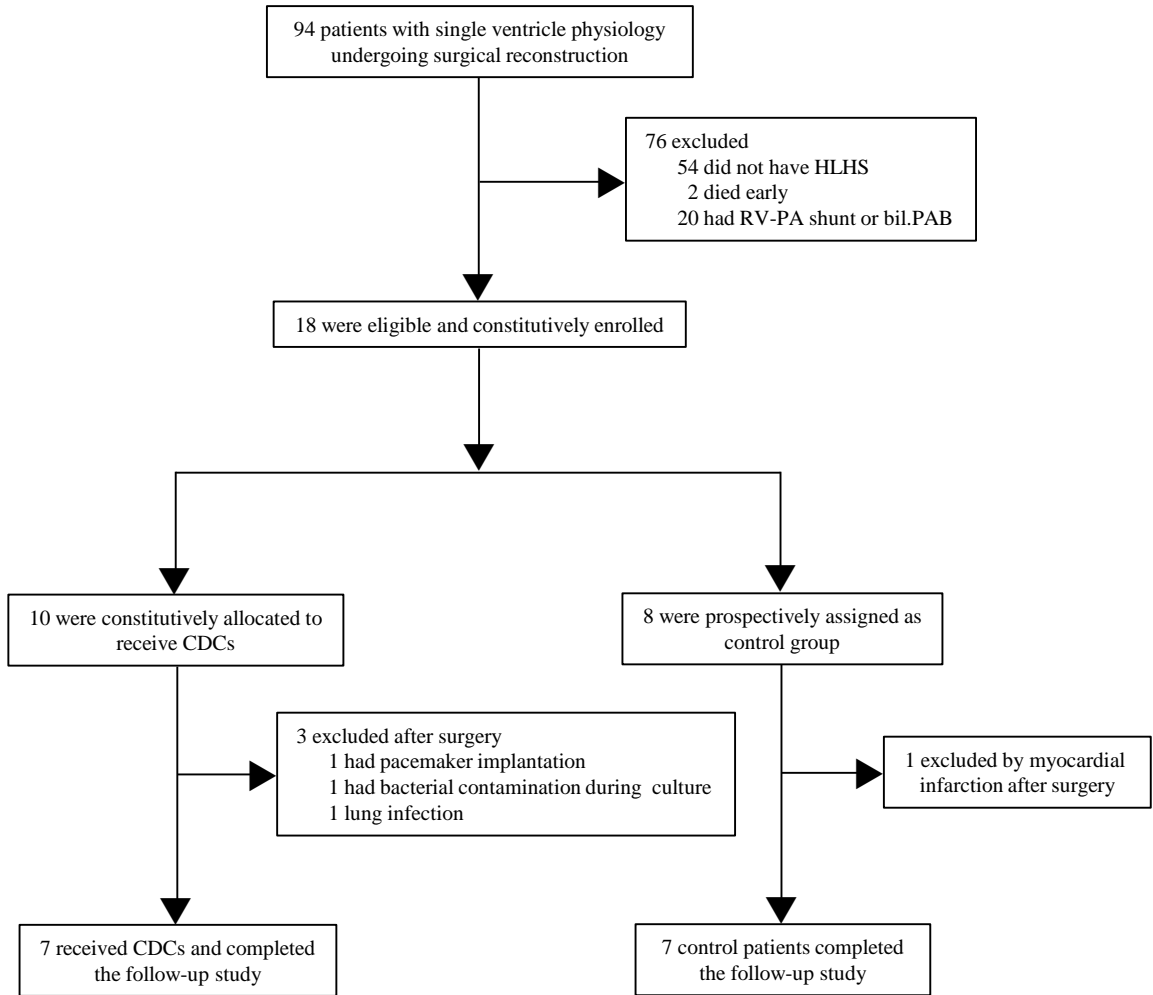
B
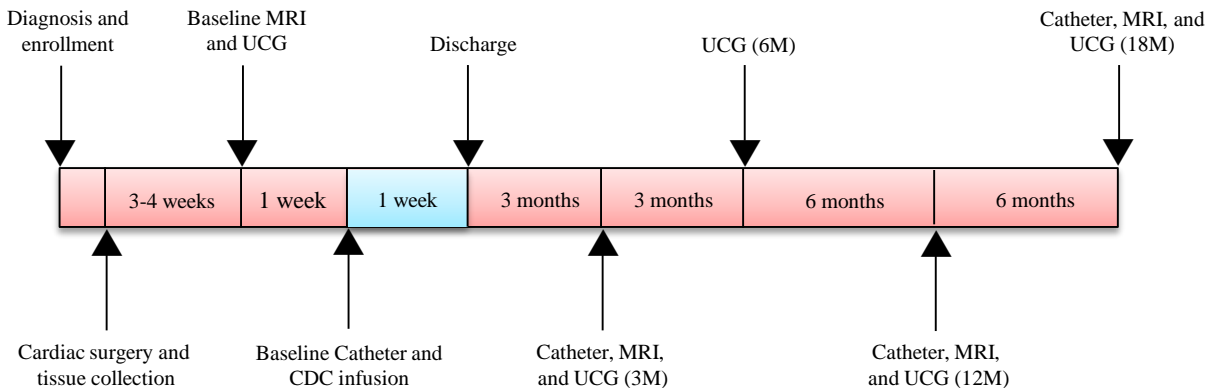
Figure 2. Prospective assignment of patients and schedule for CDC infusion.
(A) A total of 18 patients with HLHS scheduled for the second or third stage of palliation were prospectively assigned to the CDC-treated group (n=7), as well as 7 patients as control subjects. (B) cMRI, echocardiography (UCG), and angiography were scheduled for participants to analyze the effects of CDC infusion versus controls up to 18 months after treatment. The average hospitalization for CDC transfer was 8.9 ± 0.7 days. All participants completed the 18 months of follow-up examination and were analyzed.
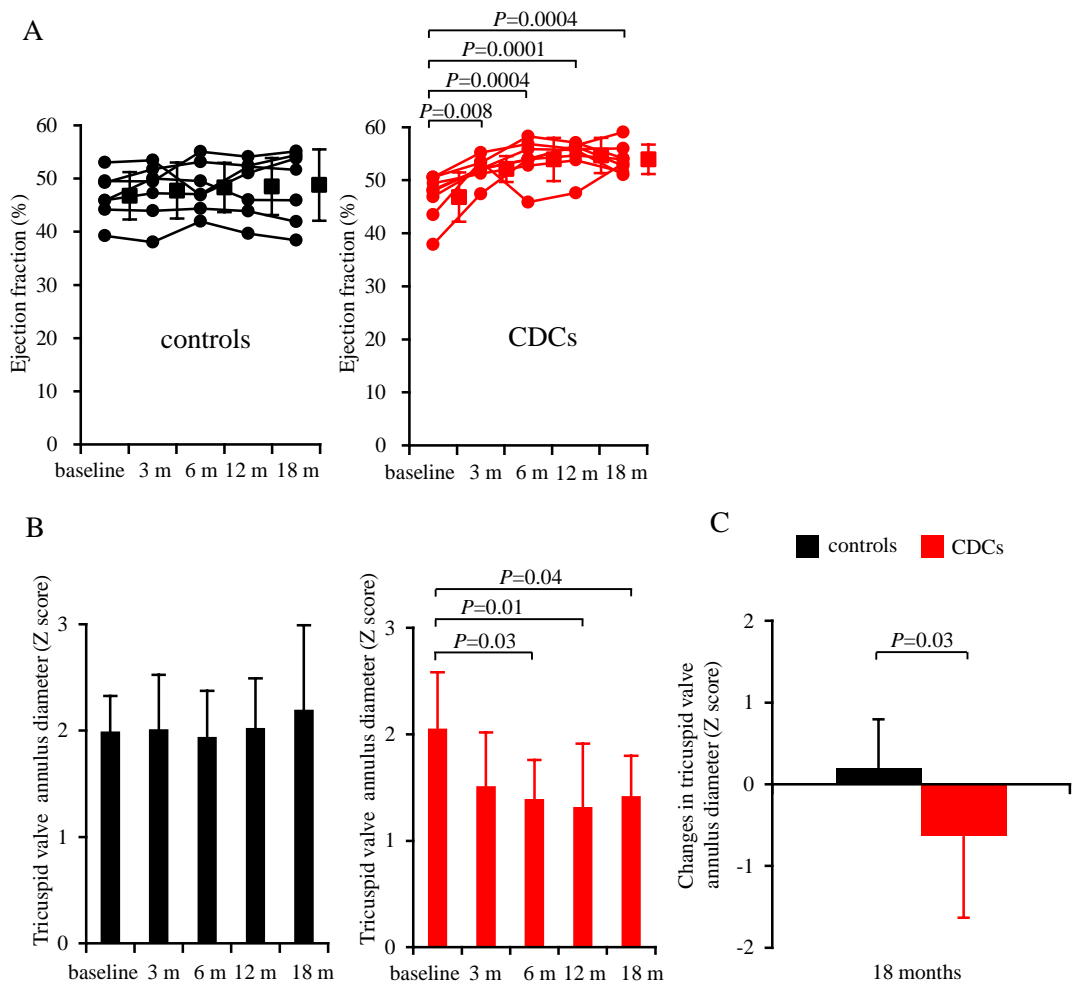
Figure 3. Endpoint analysis by echocardiography in CDC-treated patients and controls.
(A) RVEF obtained from baseline, 3, 6, 12, and 18 months after CDC transfer and control patients are shown.
(B) Tricuspid valve diameter was corrected by body surface area to achieve z-score in each measurement.
Data at baseline through 18 months of follow-up are shown and analyzed by one-way ANOVA repeated
measures and Dunnett post-hoc test. (C) Absolute changes in tricuspid valve diameter at 18 months were
analyzed using 2-tailed unpaired Student's t-test.
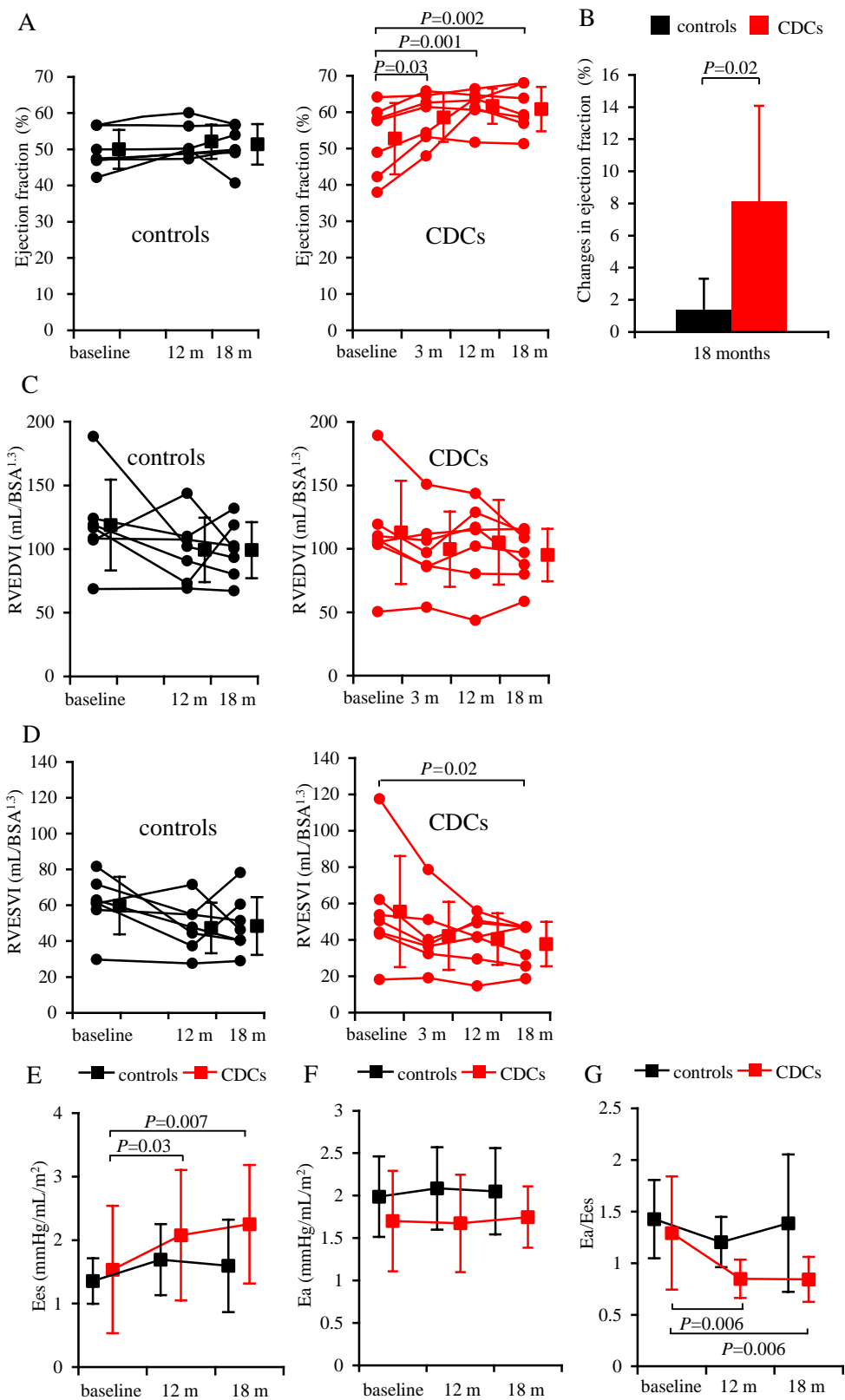
Figure 4. Angiographic assessment of cardiac function in CDC-treated and control subjects.
RVEF (A), absolute change in RVEF (B), RVEDVI (C), and RVESVI (D) measured by right ventricular angiogram from baseline to 18 months of follow-up in the 2 groups are shown. (E-G) Mechanical efficiency was approximated by pressure study and volume analysis. Ees: end-systolic elastance reflects contractility. Ea: effective arterial elastance. Ea/Ees was calculated as ventriculoarterial coupling to estimate mechanical performance. One-way ANOVA repeated measures and Dunnett post-hoc test were applied in (A and C-G). (B) was analyzed using 2-tailed unpaired Student's t-test.
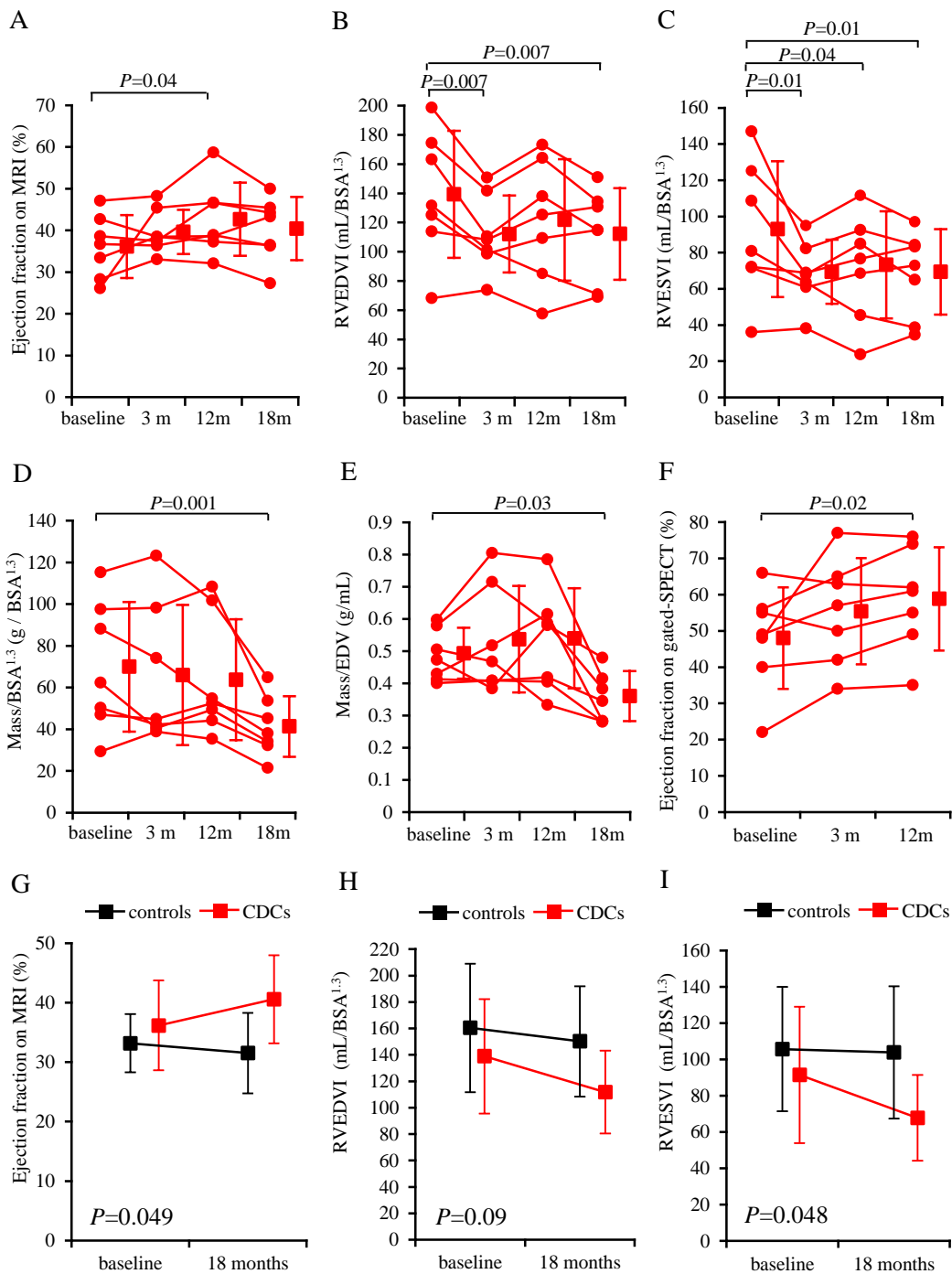
Figure 5. Cardiac function analysis by cMRI.
(A-E) Global ventricular function and assessment of volume and wall mass indexes between baseline and 18 months in CDC-treated patients are shown. (F) Gated SPECT analysis was performed before and 3 and 12 months after CDC infusion. (G-I) Global function and ventricular volume at baseline and 18 months were analyzed and compared in CDC-treated and control patients. (A-F) were analyzed by one-way ANOVA repeated measures and Dunnett post-hoc test. Two-way ANOVA were used for between groups comparison in (G-I).
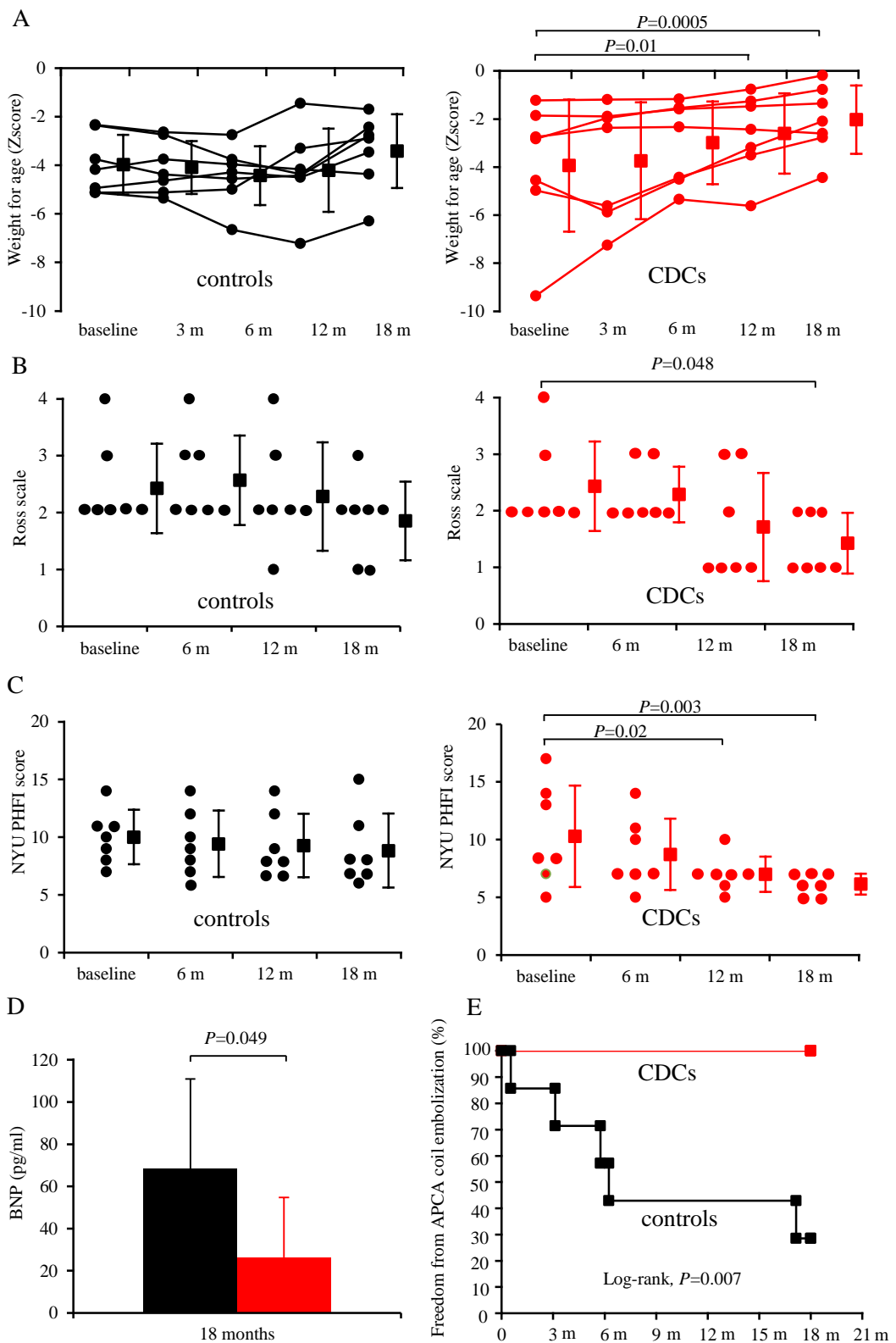
Figure 6. Functional outcome of the TICAP study.
(A) Somatic growth calculated by height and weight was converted as z-score. Heart failure status was evaluated by Ross classification (B) and NYUPHFI score (C) in all TICAP study participants during 18 months of follow-up. One-way ANOVA repeated measures and Dunnett post-hoc test were used for multiple comparisons within group in (A and C). Kruskal-Wallis with Steel post-hoc was applied in (B). (D) Serum levels of BNP at 18 months in 2 groups were analyzed by using 2-tailed unpaired Student's t-test. (E) Comparison of the freedom from coil occlusion events by intention-to-treat analysis in CDC-treated and control patients during 18-month observation.