Intracoronary Autologous Cardiac Progenitor Cell Transfer in Patients With Hypoplastic Left Heart Syndrome

The TICAP Prospective Phase 1 Controlled Trial

Shuta Ishigami, Shinichi Ohtsuki, Suguru Tarui, Daiki Ousaka, Takahiro Eitoku, Maiko Kondo, Michihiro Okuyama, Junko Kobayashi, Kenji Baba, Sadahiko Arai, Takuya Kawabata, Ko Yoshizumi, Atsushi Tateishi, Yosuke Kuroko, Tatsuo Iwasaki, Shuhei Sato, Shingo Kasahara, Shunji Sano, Hidemasa Oh

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.**

Objective: **The aim of this study was to test whether intracoronary delivery of CDCs is feasible and safe in patients with hypoplastic left heart syndrome.**

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 end point was to assess the safety, and the secondary end point included the preliminary efficacy to verify the right ventricular ejection fraction 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 right ventricular ejection fraction 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, cardiac MRI analysis of CDC-treated patients showed a higher right ventricular ejection fraction (31.5%±6.8% versus 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 seems to be feasible and safe in children with hypoplastic left heart syndrome 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: **URL: http://www.clinicaltrials.gov. Unique identifier: NCT01273857. (*Circ Res.* 2015;116:653-664. DOI: 10.1161/CIRCRESAHA.116.304671.)**

Key Words: cell therapy ■ congenital heart disease ■ hypoplastic left heart syndrome ■ stem cells

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.

Editorial, see p 566
In This Issue, see p 551

Original received June 23, 2014; revision received October 31, 2014; accepted November 14, 2014. In October, 2014, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 16 days.

From the Departments of Cardiovascular Surgery (S.I., S.T., D.O., M.O., J.K., S.A., T.K., K.Y., A.T., Y.K., S.K., S.S.), Pediatrics (S.O., T.E., M.K., K.B.), Anesthesiology and Resuscitology (T.I.), and Radiology (S.S.), Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; and Department of Regenerative Medicine, Center for Innovative Clinical Medicine, Okayama University Hospital (H.O.), Okayama, Japan.

The online-only Data Supplement is available with this article at http://circres.ahajournals.org/lookup/suppl/doi:10.1161/CIRCRESAHA.116.304671/-/DC1.

Correspondence to Hidemasa Oh, MD, PhD, Department of Regenerative Medicine, Center for Innovative Clinical Medicine, Okayama University Hospital, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan. E-mail hidemasa@md.okayama-u.ac.jp

Nonstandard Abbreviations and Acronyms	
BSA	body surface area
CDCs	cardiosphere-derived cells
cMRI	cardiac MRI
EDV	end-diastolic volume
ESV	end-systolic volume
HLHS	hypoplastic left heart syndrome
RVEF	right ventricular ejection fraction

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 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 cardiosphere-derived cells (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 effect 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 after standardized staged shunt procedures for critical patients with HLHS.

Methods

Study Design

Between January 5, 2011, and January 16, 2012, we performed a nonrandomized, 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 Online Data Supplement.

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 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, Labor and Welfare, Japan. The study was performed in accordance with the Declaration of Helsinki with written consent from all parents of eligible patients.

Study End Points

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 end points at 3 months were cardiac death caused by ventricular fibrillation, ventricular tachycardia, and myocardial infarction after CDC infusion. The secondary end points 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 end points, cardiac function at 3 months after CDC transfer and in controls at corresponding intervals was also evaluated by echocardiography, RV angiogram, and cardiac MRI (cMRI).

Cell Validation

Immunofluorescence of grown CDCs revealed that these cells expressed 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, >60% of the CDCs expressed signal-regulatory protein α and mesenchymal stem cell markers, such as CD90, CD105, and vimentin, but <1% could be detected using CD31, CD45, and DDR2 (Figure 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 , sarcoplasmic reticulum Ca^{+}-ATPase 2, and inositol trisphosphate, but remained undifferentiated by the lack of mature cardiac structural proteins, such as cardiac troponin-T, α-myosin heavy chain, myosin light chain-2v, and natriuretic peptide A (Figure 1D).

Cell Infusion

For patients allocated to receive CDCs, expanded CDCs were harvested, calculated, and prepared as 3.0×10^5 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 1 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 U 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 Iliguman type C (Fuji Systems Corporation) was selectively advanced into the coronary arteries through 0.012″ guidewire 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 minutes, whereas the progenitor cell suspensions were selectively infused into each coronary artery (1–1.5 mL by 1 infusion

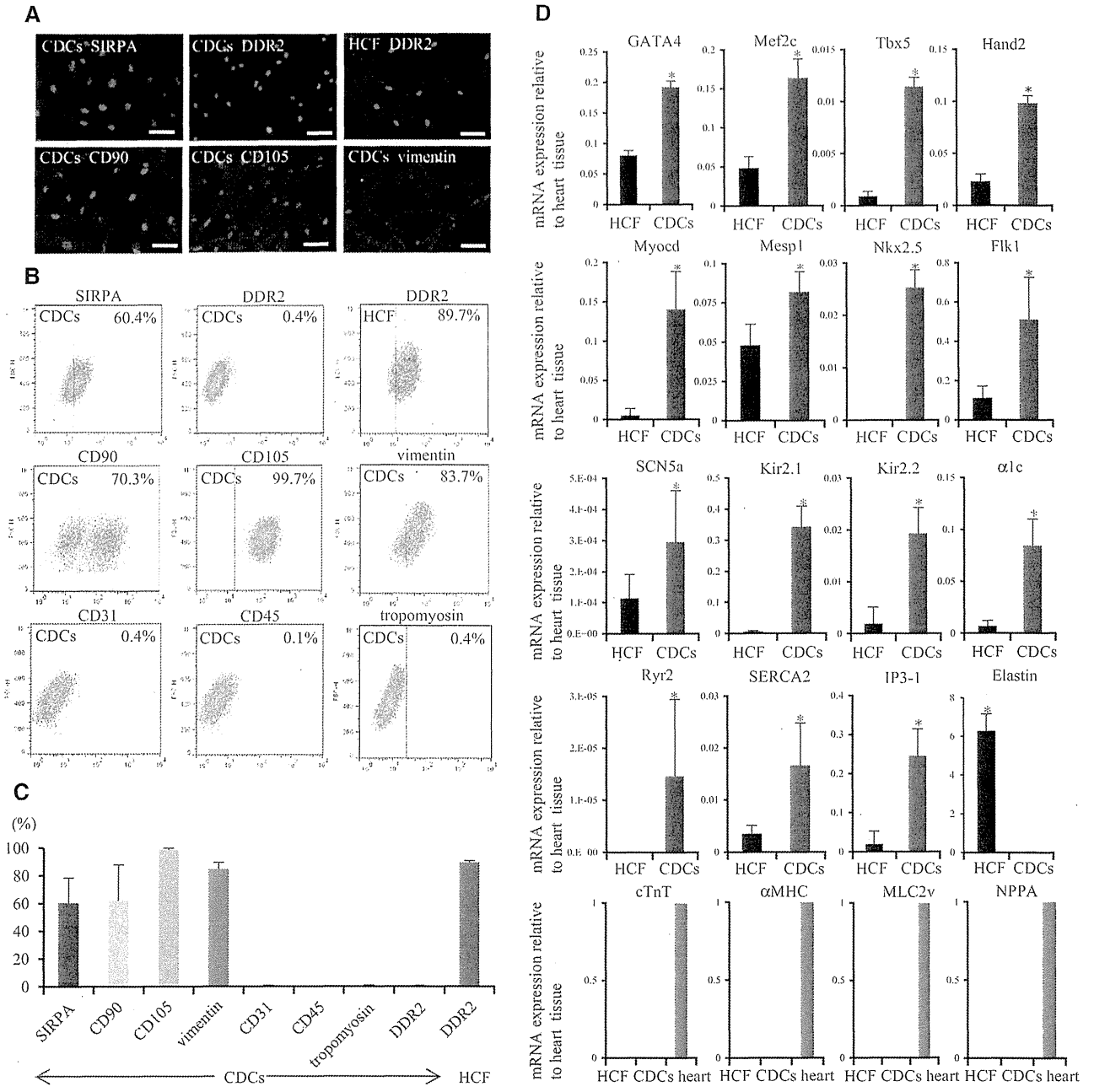

Figure 1. Cardiosphere-derived cell (CDC) processing and verification. A, Cardiospheres were mechanically selected and replated onto adherent culture to expand as CDCs. Immunofluorescence by specific antibodies was shown. Patient-derived CDCs expressed typical mesenchymal stem cell markers, CD90, CD105, and vimentin. Human cardiac fibroblasts (HCF) were used as controls for DDR2 staining. Bars, 50 mm. **B**, Fluorescence-activated cell sorting (FACS) analyses were performed to assess the quality of CDCs as characteristically consistent with cardiac progenitor cells. **C**, Summary of FACS data. CDCs expressed signal-regulatory protein α (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 t-test.

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 3 coronary arteries in 6 out of 7 patients. One patient had severely underdeveloped left circumflex branch and was treated by 2 injections. To avoid cell transfer–induced arrhythmia, amiodarone (10 μg/kg/min) was intravenously infused 30 minutes before 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 (BSA).[20]

Quantitative analysis of paired RVEF was performed, 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 E_{es} (end-systolic elastance) and E_a (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 ms; echo time, 1.6 ms; 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 $BSA^{1.5}$. 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 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 1-way analysis of variance (ANOVA) with repeated measures. No adjustments for multiple comparisons were made in safety end points evaluation.[25] Differences in mRNA expression, baseline hemodynamics, the absolute changes in tricuspid annulus diameter and RVEF, as well as serum B-type natriuretic peptide 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 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 used to adjust the type I error caused 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, 3 patients were excluded for the following reasons: 1 patient underwent pacemaker implantation during cardiac surgery and was unable to undergo cMRI examination, cell processing failure occurred in 1 patient by bacterial contamination, and 1 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 because of 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) 1 month after staged surgical procedures. Paired cMRI, angiogram, echocardiogram, and gated-single photon emission computed tomography 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 although 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 single photon emission computed tomography 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 hours and 12.1±2.8 IU/L at 48 hours 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 1 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; Figure 6E).

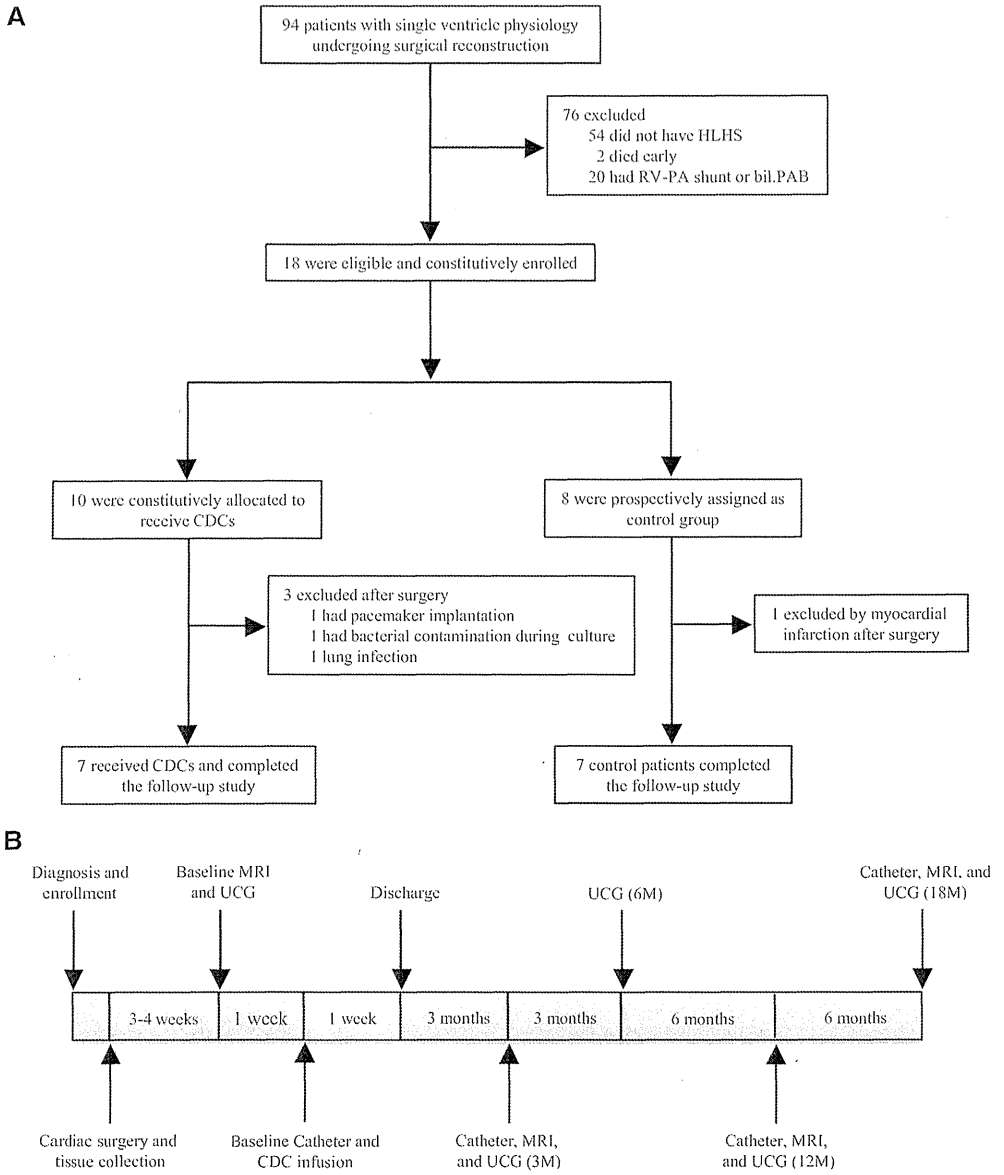

Figure 2. Prospective assignment of patients and schedule for cardiosphere-derived cell (CDC) infusion. A, A total of 18 patients with hypoplastic left heart syndrome (HLHS) scheduled for the second or third stage of palliation were prospectively assigned to the CDC-treated group (n=7), as well as 7 patients as control subjects. **B**, cardiac MRI, echocardiography (UCG), and angiography were scheduled for participants to analyze the effects of CDC infusion versus controls ≤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. PA indicates pulmonary artery; and RV, right ventricle.

Efficacy of CDC Infusion

With respect to the secondary end point, 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 end points are shown in Figure 3. Compared with baseline, cardiac function in the CDC-treated patients had significantly increased from 46.9%±4.6% to 52.1%±2.4% at 3 months (P=0.008), 53.9%±4.0% at 6 months (P=0.0004), 54.7%±3.3% at 12 months (P=0.0001), and 54.0%±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%±4.4% at baseline versus 47.7%±5.3% at 3 months; 48.3%±4.7% at 6 months; 48.5%±5.4% at 12 months; 48.7%±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

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, y	1.5±1.7	2.1±1.2	0.47
Male sex	5 (71)	4 (57)	0.50
Gestation age <38 weeks	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

ACE indicates angiotensin-converting enzyme; APCA, aorto-pulmonary collateral artery; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CDCs, cardiosphere-derived cells; n, number; PA, pulmonary artery; PDE5, phosphodiesterase type 5; RV, right ventricle; SVC, superior vena cava; TR, tricuspid valve regurgitation. 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	
Rehospitalization 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

APCA, aorto-pulmonary collateral artery; CCU, cardiac care unit; CDCs, cardiosphere-derived cells; ECMO, extracorporeal membrane oxygenation; N/A, not applicable; PLE, protein-losing enteropathy; PA, pulmonary artery; and RV, right ventricle.

*Sporadic ventricular extrasystoles were excluded.

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%±9.8% to 58.5%±6.7%; P=0.03) and this persisted at 18 months (60.8%±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 (right ventricular end-systolic volume index; 55.6±30.5 mL/BSA$^{1.5}$ at baseline to 37.8±12.2 mL/BSA$^{1.5}$ at 18 months; P=0.02), whereas the RVEDVI (right ventricular end-diastolic volume index) in both groups remained unchanged at 18 months compared with that at baseline (Figure 4C and 4D). On the basis of the cardiac catheterization data, cardiac contractility and mechanical efficiency were evaluated (Figure 4E–4G). Compared with baseline, CDC-treated patients showed a significant increase over time in end-systolic elastance measured by E_{es} (1.5±1.0 mm Hg/mL/m^2 at baseline to 2.1±1.0 mm Hg/mL/m^2 at 12 months, P=0.03; and 2.2±0.9 mm Hg/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 E_a in both groups showed no difference from baseline to 18 months later. These findings indicated an improvement of E_a/E_{es} (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%±7.5% at baseline to 42.7%±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; Figure 5B and 5C). Notably, right ventricular wall masses corrected by BSA$^{1.3}$ and EDV were significantly decreased at 18 months compared with those at baseline (Figure 5D and 5E). In addition, these long-term benefits by CDC infusion were confirmed by gated single photon emission computed tomography analysis during 12 months of follow-up (48.0%±14.0% at baseline to 58.9%±14.3% at 12 months; Figure 5F). In this study, 7 control patients underwent cMRI analysis at baseline and 18 months of follow-up (Figure 5G–5I). 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%±6.8% versus 40.4%±7.6%), which was associated with reduced RVESVI at 18 months compared with the controls (103.8±36.5 mL/BSA$^{1.3}$ versus 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 versus 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 B-type natriuretic peptide levels 18 months after CDC infusion compared with that in controls (26.3±28.5 versus 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 affect 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

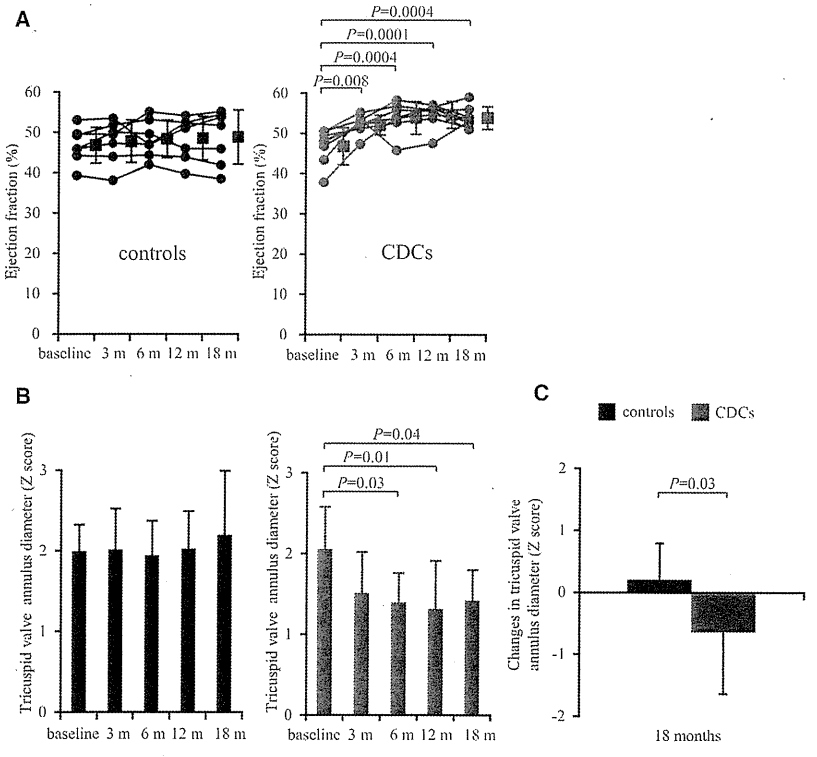

Figure 3. Endpoint analysis by echocardiography in cardiosphere-derived cell (CDC)–treated patients and controls. **A**, Right ventricular ejection fraction (RVEF) obtained from baseline, 3, 6, 12, and 18 months after CDC transfer and control patients are shown. **B**, Tricuspid valve diameter was corrected 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 1-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 t-test.

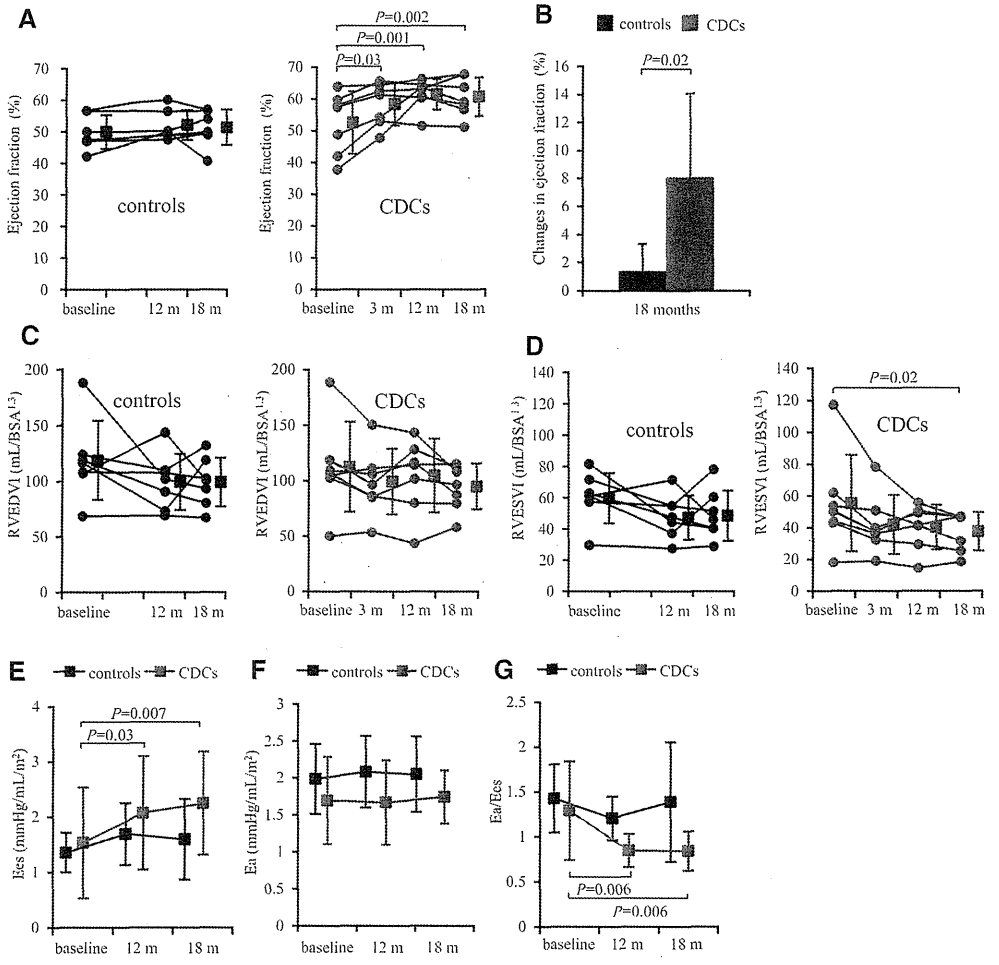

Figure 4. Angiographic assessment of cardiac function in cardiosphere-derived cell (CDC)-treated and control subjects. Right ventricular ejection fraction (RVEF; **A**), absolute change in RVEF (**B**), RVEDVI (right ventricular end-diastolic volume index; **C**), and RVESVI (right ventricular end-systolic volume index; **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. E_{es}, end-systolic elastance reflects contractility; and E_a, effective arterial elastance. E_a/E_{es} 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**, Analyzed using 2-tailed unpaired Student *t*-test.

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 B-type natriuretic peptide, as well as the decrease in functional heart failure status assessed by the New York Heart Association 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 effect 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 seem 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

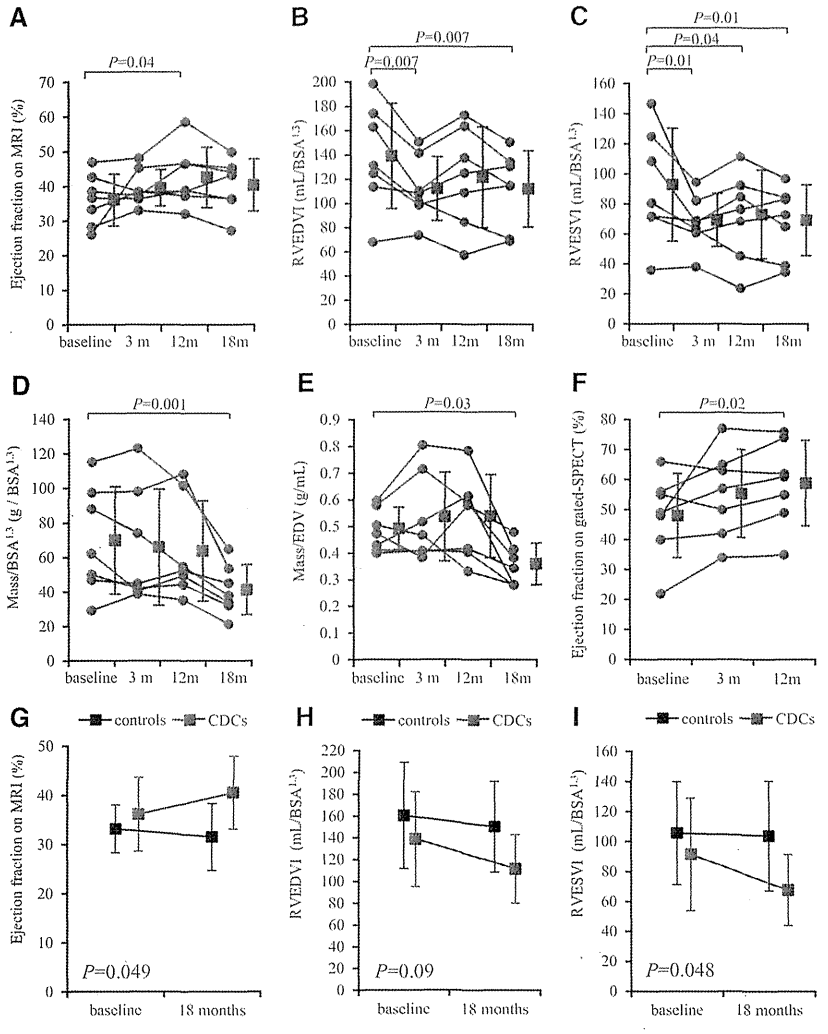

Figure 5. Cardiac function analysis by cardiac MRI (cMRI). A–E, Global ventricular function and assessment of volume and wall mass indexes between baseline and 18 months in cardiosphere-derived cell (CDC)–treated patients are shown. **F**, Gated single photon emission computed tomography (SPECT) analysis 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 1-way ANOVA repeated measures and Dunnett post hoc test. Two-way ANOVA were used for between groups comparison in **G–I**. BSA indicates body surface area.

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 preregistration 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 caused by 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 and 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 nonrandomized 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.

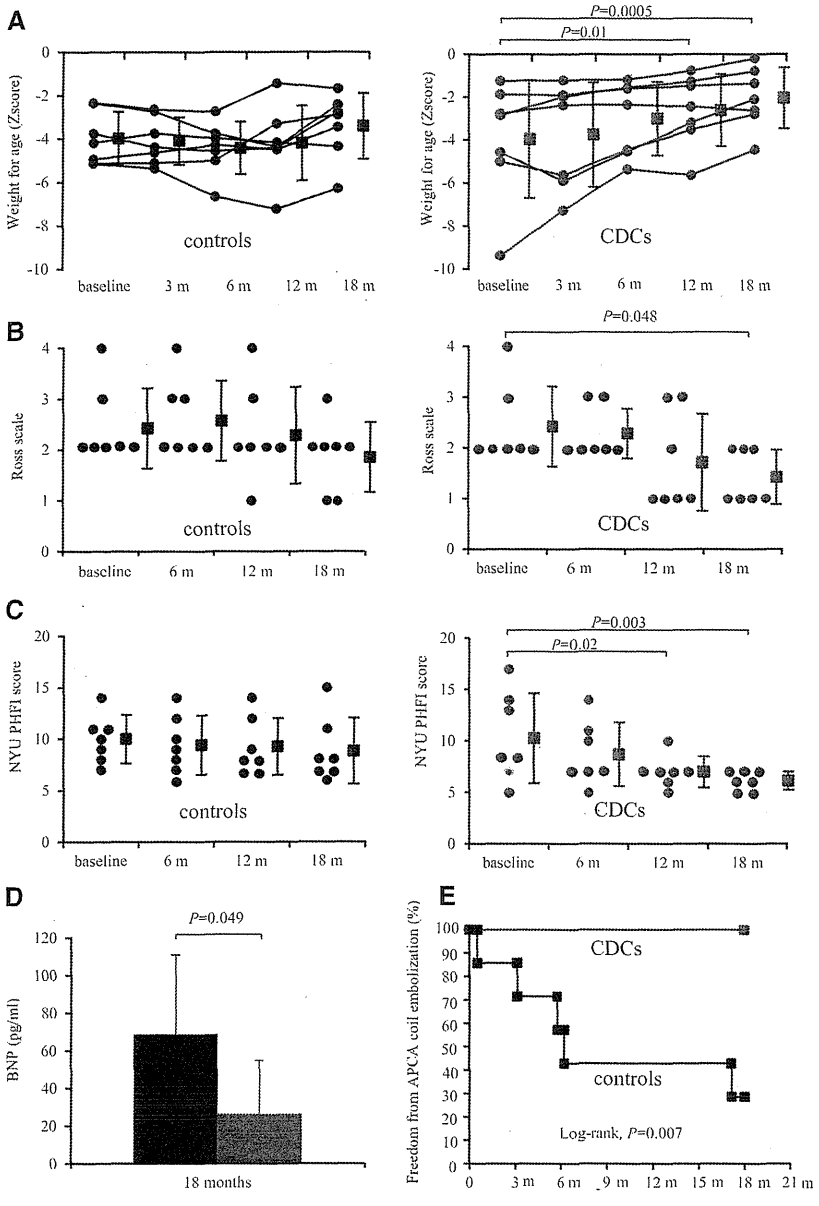

Figure 6. Functional outcome of the TICAP study. A, Somatic growth calculated by height and weight was converted as z-score. Heart failure status was evaluated by Ross classification (**B**) and NYUPHFI score (**C**) in all TICAP study participants during 18 months of follow-up. One-way ANOVA repeated 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 B-type natriuretic peptide (BNP) at 18 months in 2 groups were analyzed by using 2-tailed unpaired Student *t*-test. **E**, Comparison of the freedom from coil occlusion events by intention-to-treat analysis in cardiosphere-derived cell (CDC)–treated and control patients during 18-month observation. TICAP indicates transcoronary infusion of cardiac progenitor cells in patients with single ventricle physiology.

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, 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 ≤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.[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.

Acknowledgments

We are indebted to the patients, the parents of the patients who gave their consent to participate in this trial, and the staff of the cardiac care unit and the catheterization laboratory at Okayama University Hospital.

Sources of Funding

This study was supported by grants from the Ministry of Health, Labour and Welfare (to H. Oh), the Ministry of Education, Culture, Sports, Science and Technology (to H. Oh), and the Research Foundation of Okayama University Hospital (to S. Sano and H. Oh). The study funding source had no role in study design, data collection, data analysis, and interpretation or in the preparation of the article.

Disclosures

None.

References

1. Barron DJ, Kilby MD, Davies B, Wright JG, Jones TJ, Brawn WJ. Hypoplastic left heart syndrome. *Lancet*. 2009;374:551–564. doi: 10.1016/S0140-6736(09)60563-8.
2. Lamour JM, Kanter KR, Naftel DC, Chrisant MR, Morrow WR, Clemson BS, Kirklin JK; Cardiac Transplant Registry Database; Pediatric Heart Transplant Study. The effect of age, diagnosis, and previous surgery in children and adults undergoing heart transplantation for congenital heart disease. *J Am Coll Cardiol*. 2009;54:160–165. doi: 10.1016/j.jacc.2009.04.020.
3. Sano S, Ishino K, Kawada M, Arai S, Kasahara S, Asai T, Masuda Z, Takeuchi M, Ohtsuki S. Right ventricle-pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg*. 2003;126:504–509; discussion 509.
4. Altmann K, Printz BF, Solowiejczky DE, Gersony WM, Quaegebeur J, Apfel HD. Two-dimensional echocardiographic assessment of right ventricular function as a predictor of outcome in hypoplastic left heart syndrome. *Am J Cardiol*. 2000;86:964–968.
5. Hsu DT, Pearson GD. Heart failure in children: part I: history, etiology, and pathophysiology. *Circ Heart Fail*. 2009;2:63–70. doi: 10.1161/CIRCHEARTFAILURE.108.820217.
6. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisén J. Evidence for cardiomyocyte renewal in humans. *Science*. 2009;324:98–102. doi: 10.1126/science.1164680.
7. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA. Transient regenerative potential of the neonatal mouse heart. *Science*. 2011;331:1078–1080. doi: 10.1126/science.1200708.
8. Beohar N, Rapp J, Pandya S, Losordo DW. Rebuilding the damaged heart: the potential of cytokines and growth factors in the treatment of ischemic heart disease. *J Am Coll Cardiol*. 2010;56:1287–1297. doi: 10.1016/j.jacc.2010.05.039.
9. Hill JA, Olson EN. Cardiac plasticity. *N Engl J Med*. 2008;358:1370–1380. doi: 10.1056/NEJMra072139.
10. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*. 2003;100:12313–12318. doi: 10.1073/pnas.2132126100.
11. Tateishi K, Ashihara E, Honsho S, Takehara N, Nomura T, Takahashi T, Ueyama T, Yamagishi M, Yaku H, Matsubara H, Oh H. Human cardiac stem cells exhibit mesenchymal features and are maintained through Akt/GSK-3beta signaling. *Biochem Biophys Res Commun*. 2007;352:635–641. doi: 10.1016/j.bbrc.2006.11.096.
12. Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, Marbán E. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. 2007;115:896–908. doi: 10.1161/CIRCULATIONAHA.106.655209.
13. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marbán L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marbán E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet*. 2012;379:895–904. doi: 10.1016/S0140-6736(12)60195-0.
14. Ptaszek LM, Mansour M, Ruskin JN, Chien KR. Towards regenerative therapy for cardiac disease. *Lancet*. 2012;379:933–942. doi: 10.1016/S0140-6736(12)60075-0.
15. Zeisberg EM, Kalluri R. Origins of cardiac fibroblasts. *Circ Res*. 2010;107:1304–1312. doi: 10.1161/CIRCRESAHA.110.231910.
16. Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanty AG, Gramolini A, Keller G. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. *Nat Biotechnol*. 2011;29:1011–1018. doi: 10.1038/nbt.2005.
17. Takehara N, Tsutsumi Y, Tateishi K, Ogata T, Tanaka H, Ueyama T, Takahashi T, Takamatsu T, Fukushima M, Komeda M, Yamagishi M, Yaku H, Tabata Y, Matsubara H, Oh H. Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. *J Am Coll Cardiol*. 2008;52:1858–1865. doi: 10.1016/j.jacc.2008.06.052.
18. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Süselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM; REPAIR-AMI Investigators. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*. 2006;355:1210–1221. doi: 10.1056/NEJMoa060186.
19. Helbing WA, Bosch HG, Maliepaard C, Rebergen SA, van der Geest RJ, Hansen B, Ottenkamp J, Reiber JH, de Roos A. Comparison of echocardiographic methods with magnetic resonance imaging for assessment of right ventricular function in children. *Am J Cardiol*. 1995;76:589–594.
20. Zilberman MV, Khoury PR, Kimball RT. Two-dimensional echocardiographic valve measurements in healthy children: gender-specific differences. *Pediatr Cardiol*. 2005;26:356–360. doi: 10.1007/s00246-004-0736-z.
21. Fogel MA, Pawlowski TW, Whitehead KK, Harris MA, Keller MS, Glatz AC, Zhu W, Shore D, Diaz LK, Rome JJ. Cardiac magnetic resonance and the need for routine cardiac catheterization in single ventricle patients prior to Fontan: a comparison of 3 groups: pre-Fontan CMR versus cath evaluation. *J Am Coll Cardiol*. 2012;60:1094–1102. doi: 10.1016/j.jacc.2012.06.021.
22. Tanoue Y, Sese A, Ueno Y, Joh K, Hijii T. Bidirectional Glenn procedure improves the mechanical efficiency of a total cavopulmonary connection in high-risk fontan candidates. *Circulation*. 2001;103:2176–2180.
23. Dillman JR, Dorfman AL, Attili AK, Agarwal PP, Bell A, Mueller GC, Hernandez RJ. Cardiovascular magnetic resonance imaging of hypoplastic left heart syndrome in children. *Pediatr Radiol*. 2010;40:261–74; quiz 379. doi: 10.1007/s00247-009-1473-5.
24. Loscalzo J. Pilot trials in clinical research: of what value are they? *Circulation*. 2009;119:1694–1696. doi: 10.1161/CIRCULATIONAHA.109.861625.
25. Hare JM, Bolli R, Cooke JP, et al; Cardiovascular Cell Therapy Research Network. Phase II clinical research design in cardiology: learning the right lessons too well: observations and recommendations from the Cardiovascular Cell Therapy Research Network (CCTRN). *Circulation*. 2013;127:1630–1635. doi: 10.1161/CIRCULATIONAHA.112.000779.
26. Moyé LA, Tita AT. Defending the rationale for the two-tailed test in clinical research. *Circulation*. 2002;105:3062–3065.
27. Cabral HJ. Multiple comparisons procedures. *Circulation*. 2008;117:698–701. doi: 10.1161/CIRCULATIONAHA.107.700971.
28. Kusuoka H, Hoffman JI. Advice on statistical analysis for Circulation Research. *Circ Res*. 2002;91:662–671.
29. Eerola A, Poutanen T, Savukoski T, Pettersson K, Sairanen H, Jokinen E, Pihkala J. Cardiac troponin I, cardiac troponin-specific autoantibodies and natriuretic peptides in children with hypoplastic left heart syndrome. *Interact Cardiovasc Thorac Surg*. 2014;18:80–85. doi: 10.1093/icvts/ivt430.
30. Rogers LS, Glatz AC, Ravishankar C, Spray TL, Nicolson SC, Rychik J, Rush CH, Gaynor JW, Goldberg DJ. 18 years of the Fontan operation

at a single institution: results from 771 consecutive patients. *J Am Coll Cardiol*. 2012;60:1018–1025. doi: 10.1016/j.jacc.2012.05.010.
31. Tateishi K, Takehara N, Matsubara H, Oh H. Stemming heart failure with cardiac- or reprogrammed-stem cells. *J Cell Mol Med*. 2008;12:2217–2232. doi: 10.1111/j.1582-4934.2008.00487.x.
32. Bernstein HS, Srivastava D. Stem cell therapy for cardiac disease. *Pediatr Res*. 2012;71:491–499. doi: 10.1038/pr.2011.61.
33. Rupp S, Zeiher AM, Dimmeler S, Tonn T, Bauer J, Jux C, Akintuerk H, Schranz D. A regenerative strategy for heart failure in hypoplastic left heart syndrome: intracoronary administration of autologous bone marrow-derived progenitor cells. *J Heart Lung Transplant*. 2010;29:574–577. doi: 10.1016/j.healun.2009.10.006.
34. Rupp S, Jux C, Bönig H, Bauer J, Tonn T, Seifried E, Dimmeler S, Zeiher AM, Schranz D. Intracoronary bone marrow cell application for terminal heart failure in children. *Cardiol Young*. 2012;22:558–563. doi: 10.1017/S1047951112000066.
35. Lee ST, White AJ, Matsushita S, Malliaras K, Steenbergen C, Zhang Y, Li TS, Terrovitis J, Yee K, Simsir S, Makkar R, Marbán E. Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in pigs with heart failure post-myocardial infarction. *J Am Coll Cardiol*. 2011;57:455–465. doi: 10.1016/j.jacc.2010.07.049.
36. Li TS, Cheng K, Malliaras K, Smith RR, Zhang Y, Sun B, Matsushita N, Blusztajn A, Terrovitis J, Kusuoka H, Marbán L, Marbán E. Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere-derived cells. *J Am Coll Cardiol*. 2012;59:942–953. doi: 10.1016/j.jacc.2011.11.029.
37. Frommelt PC, Guey LT, Minich LL, et al; Pediatric Heart Network Investigators. Does initial shunt type for the Norwood procedure affect echocardiographic measures of cardiac size and function during infancy?: the Single Vventricle Reconstruction trial. *Circulation*. 2012;125:2630–2638. doi: 10.1161/CIRCULATIONAHA.111.072694.
38. Rathod RH, Prakash A, Powell AJ, Geva T. Myocardial fibrosis identified by cardiac magnetic resonance late gadolinium enhancement is associated with adverse ventricular mechanics and ventricular tachycardia late after Fontan operation. *J Am Coll Cardiol*. 2010;55:1721–1728. doi: 10.1016/j.jacc.2009.12.036.
39. Simpson DL, Mishra R, Sharma S, Goh SK, Deshmukh S, Kaushal S. A strong regenerative ability of cardiac stem cells derived from neonatal hearts. *Circulation*. 2012;126:S46–S53. doi: 10.1161/CIRCULATIONAHA.111.084699.
40. de Jong R, Houtgraaf JH, Samiei S, Boersma E, Duckers HJ. Intracoronary stem cell infusion after acute myocardial infarction: a meta-analysis and update on clinical trials. *Circ Cardiovasc Interv*. 2014;7:156–167. doi: 10.1161/CIRCINTERVENTIONS.113.001009.
41. Anderson PA, Sleeper LA, Mahony L, et al; Pediatric Heart Network Investigators. Contemporary outcomes after the Fontan procedure: a Pediatric Heart Network multicenter study. *J Am Coll Cardiol*. 2008;52:85–98. doi: 10.1016/j.jacc.2008.01.074.
42. Newburger JW, Sleeper LA, Frommelt PC, et al; Pediatric Heart Network Investigators. Transplantation-free survival and interventions at 3 years in the single ventricle reconstruction trial. *Circulation*. 2014;129:2013–2020. doi: 10.1161/CIRCULATIONAHA.113.006191.
43. Bellsham-Revell HR, Tibby SM, Bell AJ, Witter T, Simpson J, Beerbaum P, Anderson D, Austin CB, Greil GF, Razavi R. Serial magnetic resonance imaging in hypoplastic left heart syndrome gives valuable insight into ventricular and vascular adaptation. *J Am Coll Cardiol*. 2013;61:561–570. doi: 10.1016/j.jacc.2012.11.016.
44. McCrindle BW, Zak V, Sleeper LA, Paridon SM, Colan SD, Geva T, Mahony L, Li JS, Breitbart RE, Margossian R, Williams RV, Gersony WM, Atz AM; Pediatric Heart Network Investigators. Laboratory measures of exercise capacity and ventricular characteristics and function are weakly associated with functional health status after Fontan procedure. *Circulation*. 2010;121:34–42. doi: 10.1161/CIRCULATIONAHA.109.869396.

Novelty and Significance

What Is Known?

- Hypoplastic left heart syndrome 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 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 hypoplastic left heart syndrome 1 month after staged palliations.
- Patient-derived CDCs could improve right ventricular function from 3 to 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.

We conducted a clinical trial to assess the feasibility and safety of intracoronary injection of cardiac progenitor cells to treat the critical congenital heart defects, such as hypoplastic left heart syndrome in children. The safety concerns were monitored by cardiac enzyme measurement, Holter ECG, cMRI, and single photon emission computed tomography analysis during cell infusion with 18 months of follow-up. There were no reports of proarrhythmic 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. Results of this proof-of-concept study suggest 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. These findings provide a 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.

Supplemental Material

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

Shuta Ishigami, M.D.[1], Shinichi Ohtsuki, M.D., Ph.D.[2], Suguru Tarui, M.D.[1], Daiki Ousaka, R.D.C.S.[1], Takahiro Eitoku, M.D.[2], Maiko Kondo, M.D.[2], Michihiro Okuyama, M.D.[1], Junko Kobayashi, M.D.[1], Kenji Baba, M.D., Ph.D.[2], Sadahiko Arai, M.D.,Ph.D.[1], Takuya Kawabata, M.D., Ph.D.[1], Ko Yoshizumi, M.D., Ph.D.[1], Atsushi Tateishi, M.D., Ph.D.[1], Yosuke Kuroko, M.D.[1], Ph.D., Tatsuo Iwasaki, M.D., Ph.D.[3], Shuhei Sato, M.D., Ph.D.[4], Shingo Kasahara, M.D.Ph.D.[1], Shunji Sano, M.D., Ph.D.[1], and Hidemasa Oh, M.D., Ph.D.[5,*]

Departments of Cardiovascular Surgery[1], Pediatrics[2], Anesthesiology and Resuscitology[3], and Radiology[4], Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences; Department of Regenerative Medicine[5], Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama, Japan

Table of Contents

Study Protocol

A) Study Population and Methods

The study population is patients with hypoplastic left heart syndrome or cardiac failure with functional single-ventricle physiology. Cardiac tissue (100 to 250 mg) is collected from the right atrium during conventional surgical repair, and autologous cardiac-derived progenitor cells (CDCs) are established. Cardiac progenitor cells are grown for approximately 2 to 3 weeks up to the cell number required for transplantation and are transplanted by direct intracoronary injection during cardiac catheterization test for post-cardiac surgical examination. The patients are followed up for one year after the cell transplantation for safety evaluation.

B) Study Endpoints

The primary endpoint of this phase 1 study is to assess the safety and feasibility of intracoronary injection of cardiac progenitor cells in patients with single-ventricle physiology. The evaluation of safety includes major cardiac events as shown below for which a causal relationship to the therapy cannot be ruled out.

- Cardiac-event-related death including cardiac arrest
- Ventricular tachycardia or fibrillation
- Re-hospitalization for heart failure
- New event of acute coronary syndrome
- Cardiac tamponade or pericarditis

The secondary endpoint includes all procedure-related events and complications that are not covered by the items above.

C) Quality Control of Human Cardiac Progenitors

The methods for cell purification are in accordance with Good Manufacturing Practice (GMP) guidelines and the whole process of primary separation of progenitor cells, intermediate culture, and final amplification and purification is conducted in the Facility for Translational Medicine, which conforms to GMP and is located in the Okayama University Hospital establishment.

To confirm that the cells isolated from autologous cardiac tissue have characteristics of cardiac progenitor cells, the following transcription factors and expression markers, which are shared among cardiac progenitor cells, are evaluated as indicated.

- The transcription factors specific to cardiac muscle, Nkx2.5 and GATA4, are positive.
- Mesenchymal stem cell markers, CD90, CD105, and SIRPA, are positive, but CD45 and MHC class II, an antigen for immune presentation, are negative.

For verification of the proliferation capacity, cells are incubated as usual after isolation and, if the number of cells does not reach the specified level (3.0×10^5 cells/kg) after approximately

21 days, the isolated nucleated cells are considered to lack proliferative capacity. If survival is not 50% or more after cells are stored frozen and then thawed, the frozen cells are considered to have no viability.

The safety of the cells used for transplantation is ensured by the analysis results of karyotyping and tumor formation analysis by inoculation in immune-deficient mice, both of which have been conducted in a preclinical study, as a nonclinical study. In addition, karyotyping analysis has been conducted in all transplanted patients in the TICAP Phase I Study to confirm the reproducibility of safety quality assurance.

The infection of the cells during the culture process is verified by bacterial culture, fungal culture, mycoplasma test, endotoxin assay, and viral test of culture supernatant when cell culture is started and when cells are frozen, thawed, and released.

D) Diagnostic Criteria

Pure single-ventricle physiology with only a single ventricle is clinically extremely rare, and there is often a small secondary ventricle. In terms of function, however, most secondary ventricles show an accessory morphology that lacks sufficient power to eject blood effectively to the systemic and pulmonary blood flow. The cardiovascular physiology of functional single-ventricle physiology is defined by factors such as the outlet, inlet, and the shunt flow through the hole caused by atrial septal defect, systemic and pulmonary venous return, pulmonary vascular resistance, and atrioventricular valve regurgitation. The systemic flow is maintained by the right-to-left shunt and the arterial duct in the case of systemic outflow tract obstruction and by the left-to-right shunt and the arterial duct in the case of pulmonary outflow tract obstruction.

Morphological evaluation by echocardiography is the most sensitive and useful test. There are usually two separate atrioventricular valves in the double-inlet left ventricle (DILV), and one of them is associated with atresia, stenosis, or regurgitation. A pentacuspid valve is often observed in a type of single-ventricle physiology with a common atrioventricular valve. In a single-ventricle physiology of left ventricular type, the aorta is often formed from the hypoplastic right ventricle, and the right and left pulmonary arteries branch off from the left ventricle.

Another useful method for laboratory diagnosis is the cardiac catheterization test. The cardiac catheterization test mainly consists of 1) anatomical analysis based on hemodynamics and systemic and pulmonary blood flow and 2) detailed analysis of the connection between the aorta and the outflow tract from the atrioventricle and ventricle, ventricular morphology and function, pulmonary vascular resistance, deviation in the course of the aorta, and pulmonary artery tract in the whole body. One of the most important analytical factors in cardiac

catheterization is the measurement of pulmonary vascular resistance. Additionally, the collateral circulation from the aorta to the pulmonary artery is confirmed by pulmonary arteriography, and the pulmonary blood flow is controlled by coil embolization in clinical practice.[1]

E) Eligibility Criteria

The patients who have functional single-ventricle physiology with cardiac failure and have an indication of stage 2 (Glenn) or stage 3 (Fontan) surgery, meet the criteria below, and do not meet any of the exclusion criteria below are enrolled as eligible subjects. A maximum of 7 consecutive patients will be assigned to the treatment arm to receive intracoronary infusion of cardiac progenitor cells followed by 7 consecutive patients in the control arm.

- Age between 0 and 6 years at the time of patient enrollment
- Patients who have received a written explanation of participation in the study and have given written consent

F) Exclusion Criteria

- Cardiogenic shock
- A patient dependent on extracorporeal circulation
- A patient with lethal, uncontrollable arrhythmia
- A patient with a complication of coronary artery disease
- A patient with a complication of brain dysfunction due to circulatory failure
- A patient with malignant neoplasm
- A patient with a complication of serious neurologic disorder
- A patient with high-grade pulmonary embolism or pulmonary hypertension
- A patient with high-grade renal failure
- A patient with multiple organ failure
- Active infection (including endocarditis)
- Sepsis
- Active hemorrhagic disease (e.g. gastrointestinal bleeding, injury)
- Inability to complete the protocol treatment and baseline to follow-up examinations

G) Progenitor Cell Infusion

As a control angiogram, a 5 French sheath is inserted from the right lower leg artery under general anesthesia. Coronary angiography is performed on the basis of the preoperative diagnosis, and the course of dominant coronary artery toward the functional ventricle and the presence or absence of mass formation and organic stenosis have to be confirmed. The successive protocol treatment is conducted in the assigned patients as follows.

- Administer a saturating bolus dose (100 unit/kg) of heparin in the early stage when catheterization test is conducted
- Transplant the progenitor cells using a perfusion balloon catheter so that the transplanted progenitor cells will adhere to and penetrate into the cardiac artery
- Suspend 3.0×10^5 cells/kg autologous cardiac progenitor cells in a total of 3 mL of cell culture medium containing 10% autologous serum
- Separate the cells into three 1-mL doses for three coronary arteries; the time of injection per dose is 2 to 3 minutes
- Inject the cardiac progenitor cells through the balloon inflated at a low pressure
- After each progenitor cell injection, place a 3-minute interval of coronary reflow to minimize the ischemia in cardiac muscles

H) Termination Criteria for Protocol Treatment

If the following items occur, the protocol treatment is terminated and the date and reason for termination are recorded in the case report form.

- When the subject withdraws consent to participate in the study, when the subject's consciousness becomes impaired, or when a confused state develops in which the will to continue the study cannot be expressed
- When the subject is found ineligible after enrollment
- When cardiac progenitor cells cannot be collected
- When the number of cells does not reach the required level (3.0×10^5 cells/kg) within 21 days of incubation during the cardiac progenitor cell culture process
- A test for endotoxin, bacteria, fungi, mycoplasma, or virus in the cell supernatant is positive when culture is started, when cells are frozen, and when cells are thawed
- When an invasive treatment (such as use of a ventricular assist device, ventricular resynchronization therapy, or heart transplantation) is required during the waiting period of the study therapy and during the follow-up period after the study therapy (excluding the implantation of a transient pacemaker and the use of a ventricular assist device in the intensive care unit during the postoperative recovery period)
- When a lethal complication (including ventricular fibrillation) occurs during cell transplantation therapy
- When the subject dies
- Other occasions, such as when the investigator considers that the protocol treatment should be terminated to ensure the safety of the subject and to respect the patient's right of self-determination

I) Safety Evaluation

Adverse events refer to all unfavorable or unintended signs (including abnormal laboratory values), symptoms, and diseases that occur from the start of protocol treatment to two years after cell infusion irrespective of the causal relationship to the corresponding treatment. Serious adverse events (SAE) refer to the following adverse events.

- Death
- Events that can result in death
- Events requiring hospitalization for treatment or prolongation of hospitalization
- Heart failure
- Ventricular and vascular perforation due to catheterization
- Acute myocardial ischemia during cell infusion
- When bacteria are detected in culture supernatant and infection with the same bacteria occurs at the site of operation
- Tumorigenesis from the site of cell transplantation
- Ventricular arrhythmia that occurs upon intracoronary injection for cell transplantation
- Atrioventricular block
- Cerebrovascular accident: cerebral infarction, intracerebral hemorrhage
- Respiratory insufficiency: atelectasis, pneumothorax, and pneumonia
- Abnormal hepatic functions: elevations in GOT, GPT, ALP, and LDH
- Abnormal renal functions: elevation in serum creatine, renal failure
- Hemorrhage: wound hemorrhage, cardiac tamponade
- Other events as serious as those listed above

J) Data Monitoring

The chair of the Independent Data Monitoring Committee deliberates on the following items regularly at the indicated frequencies.

- Study progress report by the principal investigator (every 6 months)
- Report of the results of interim analysis from the statistical analysis manager
- Report on serious adverse events sent from the principal investigator
- If the incidence of adverse events is considerably larger than the initial expectation, evaluation of the items that are considered to be the cause of this
- The impact of important information newly obtained in the clinical study on the continuation of the entire clinical study
- Relevant reports not associated with this study, such as papers and conference presentations
- When the principal investigator, the lead investigator, or the chair of the Independent Data Monitoring Committee considers that deliberation is necessary

Methods

Safety Monitoring

The study subjects were closely monitored by an independent data monitoring committee at the Center for Innovative Clinical Medicine. Myocardial ischemia and perfusion were monitored by cardiac enzyme examination, ECG recording, Holter monitoring, and gated SPECT analysis at baseline through follow-up period. The serious adverse events were reported to institutional and independent monitoring committees and transmitted to the Ministry of Health, Labour and Welfare in accordance with the safety evaluation protocol. The study termination criteria were developed in consultation with the specified institutional committee members at Okayama University Hospital. The Center for Innovative Clinical Medicine was responsible for coordination of the collection, standardization, integration, and analysis of study data as well as the preparation and distribution of the required reports to corresponding clinical investigators.

Cell Processing

The atrial tissues were obtained during cardiac surgery, yielding an average 95 ± 23 mg per sample. The cell purification was performed in Good Manufacturing Practice facility in Okayama University Hospital. The specimens were excised, minced, and digested with 0.4% type II collagenase and 0.01% DNAse. Obtained cells were then plated at 20 cells/mL in ultra-low culture dishes as a floating culture system to generate cardiospheres with growth medium containing DMEM/F12, 10% autologous serum, 20 ng/mL epidermal growth factor (Sigma), and 40 ng/mL basic fibroblast growth factor (Promega). Cardiospheres were selectively picked and dissected into single cells to obtain CDCs by exposure to a 0.05% Trypsin/EDTA solution and grown as an adherent culture.[2] The specified cell dose could be achieved by three passages within 2 weeks of tissue sampling. Human cardiac fibroblasts were purchased from Lonza.

Immunofluorescence and Flow Cytometry

To process the individual manufactured CDCs, grown cells were labeled with the following antibodies: mouse monoclonal or rabbit polyclonal antibodies against SIRPA (BioLegend), DDR2, CD31, CD45 (abcam), CD90, CD105 (BD Biosciences), vimentin (PROGEN), and tropomyosin (Sigma). For immunofluorescence, cells were fixed in 4% paraformaldehyde and were stained with primary mouse monoclonal antibodies. Secondary antibodies were conjugated to Alexa Flour 546 anti-mouse IgG (Invitrogen), and nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI, Molecular Probes). Images were captured with a BZ-8000 (Keyence) and IX71 (Olympus Corporation). For flow cytometric analysis, FITC-goat anti-mouse IgG/IgM or FITC-goat anti-rabbit IgG polyclonal antibody (Pharmingen) was used