Cell Therapy Trials in Congenital Heart Disease
Hidemasa Oh

Abstract: Dramatic evolution in medical and catheter interventions and complex surgeries to treat children with congenital heart disease (CHD) has led to a growing number of patients with a multitude of long-term complications associated with morbidity and mortality. Heart failure in patients with hypoplastic left heart syndrome predicated by functional single ventricle lesions is associated with an increase in CHD prevalence and remains a significant challenge. Pathophysiological mechanisms contributing to the progression of CHD, including single ventricle lesions and dilated cardiomyopathy, and adult heart disease may inevitably differ. Although therapeutic options for advanced cardiac failure are restricted to heart transplantation or mechanical circulatory support, there is a strong impetus to develop novel therapeutic strategies. As lower vertebrates, such as the newt and zebrafish, have a remarkable ability to replace lost cardiac tissue, this intrinsic self-repair machinery at the early postnatal stage in mice was confirmed by partial ventricular resection. Although the underlying mechanistic insights might differ among the species, mammalian heart regeneration occurs even in humans, with the highest degree occurring in early childhood and gradually declining with age in adulthood, suggesting the advantage of stem cell therapy to ameliorate ventricular dysfunction in patients with CHD. Although effective clinical translation by a variety of stem cells in adult heart disease remains inconclusive with respect to the improvement of cardiac function, case reports and clinical trials based on stem cell therapies in patients with CHD may be invaluable for the next stage of therapeutic development. Dissecting the differential mechanisms underlying progressive ventricular dysfunction in children and adults may lead us to identify a novel regenerative therapy. Future regenerative technologies to treat patients with CHD are exciting prospects for heart regeneration in general practice. (Circ Res. 2017;120:1353-1366. DOI: 10.1161/CIRCRESAHA.117.309697.)

Key Words: dilated cardiomyopathy ■ general practice ■ hypoplastic left heart syndrome ■ prevalence ■ stem cell

Patients with congenital heart disease (CHD), particularly those with hypoplastic left heart syndrome (HLHS) and related single ventricle physiology, are characterized by an increased ventricular workload resulting in cardiac dysfunction.1 Despite the recent evolution in medical care, catheter interventions and surgical procedures have increased substantially over the past 2 decades, creating a growing population of children and adults with CHD that mandates new treatment strategies to optimize the long-term outcomes.2 Although heart transplantation remains the ultimate surgical option for eligible patients with advanced cardiac failure, evidence for advanced cardiac failure are restricted to heart transplantation or mechanical circulatory support, there is a strong impetus to develop novel therapeutic strategies. As lower vertebrates, such as the newt and zebrafish, have a remarkable ability to replace lost cardiac tissue, this intrinsic self-repair machinery at the early postnatal stage in mice was confirmed by partial ventricular resection. Although the underlying mechanistic insights might differ among the species, mammalian heart regeneration occurs even in humans, with the highest degree occurring in early childhood and gradually declining with age in adulthood, suggesting the advantage of stem cell therapy to ameliorate ventricular dysfunction in patients with CHD. Although effective clinical translation by a variety of stem cells in adult heart disease remains inconclusive with respect to the improvement of cardiac function, case reports and clinical trials based on stem cell therapies in patients with CHD may be invaluable for the next stage of therapeutic development. Dissecting the differential mechanisms underlying progressive ventricular dysfunction in children and adults may lead us to identify a novel regenerative therapy. Future regenerative technologies to treat patients with CHD are exciting prospects for heart regeneration in general practice. (Circ Res. 2017;120:1353-1366. DOI: 10.1161/CIRCRESAHA.117.309697.)

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revealed a remarkable regenerative capability compared with those from adults in experimental myocardial infarction, suggesting a possible contribution of CDCs in congenital cardiac repair. The biological significance of young patient–derived c-KIT–expressing CPCs were investigated by direct comparison with neonatal-CPCs versus adult-CPCs in in vitro and in vivo experiments, and the plausible mechanisms were unveiled by a series of deep proteomic analyses. In addition, CDC-derived exosomes have emerged as a key component to interpret the cardiac function improvement seen in experimental myocardial infarction, suggesting a possible contribution of CDCs in congenital cardiac repair. The biological significance of young patient–derived c-KIT–expressing CPCs were investigated by direct comparison with neonatal-CPCs versus adult-CPCs in in vitro and in vivo experiments, and the plausible mechanisms were unveiled by a series of deep proteomic analyses. In addition, CDC-derived exosomes have emerged as a key component to interpret the cardiac function improvement seen in experimental myocardial infarction, suggesting a possible contribution of CDCs in congenital cardiac repair.

**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>CDCs</td>
<td>cardiosphere-derived cells</td>
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<tr>
<td>CHD</td>
<td>congenital heart disease</td>
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<tr>
<td>CPCs</td>
<td>cardiac progenitor cells</td>
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<td>DCM</td>
<td>dilated cardiomyopathy</td>
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<td>HIFs</td>
<td>hypoxia-inducible factors</td>
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<td>HLHS</td>
<td>hypoplastic left heart syndrome</td>
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<td>MSCs</td>
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<td>RV-PAS</td>
<td>right ventricle to pulmonary artery shunt</td>
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**Mortality From CHD**

The incidence of CHD based on diagnosis in newborns is estimated to be between 3 and 20 per 1000 live births. Survival in children with CHD has dramatically improved over the past few decades because of significant surgical advances. Recent meta-regression analysis has also shown that a more recent registry period was closely associated with increased 1- to 10-year survival. In contrast, the mortality rate in patients with CHD was greatest during the first year of life, and the cumulative survival decreased gradually during childhood to adulthood.

Mortality resulting from CHD declined overall when compared with that of past decade. Analysis of age-specific mortality caused by CHD has shown that infant mortality is highest and lowest among children of 5 and 17 years of age, respectively. There was a marked increase in mortality among adults >65 years of age, which may be caused by an increase in CHD prevalence in the general population since 1985. The epidemic growth of CHD could also be seen in lifetime estimates of CHD prevalence using population-based data sources. The prevalence estimates indicate that there is a significant increase in CHD prevalence in adult patients, which account for more than 60% of the entire population with CHD. Although mortality resulting from CHD has decreased, the impact of our expanding population and long-term survivors in children and adults with CHD may lead to an increase in patients with heart failure, which is the leading cause of death after cardiovascular surgery. The increased incidence of heart failure may require repeated hospitalization due to the significant morbidity, and may increase the risk of mortality.

**Single Ventricle Reconstruction Trial**

As described above, CHD mortality is highest among infants, and the median age of death resulting from CHD has been reported to be 1 year. This trend did not change during the study period, and patients with HLHS (<1 year of age) showed the highest mortality compared with that of other CHD diagnoses. Patients with HLHS and related single ventricle lesions are essentially fatal and require immediate structural repair by 3-stage surgical reconstructions. Typical presentation of morphological abnormalities in single ventricle physiology includes inadequately developed 1 ventricle with systemic outflow obstruction. Both systemic and pulmonary blood flow are supplied by single functional ventricle, which is not sufficient to support systemic circulation.

The first stage of palliation for infants with single ventricle physiology is the Norwood procedure, in which pulmonary blood flow is supplied by either a modified Black–Taussig shunt or a right ventricle to pulmonary artery shunt (RV-PAS). The RV-PAS is considered to have the advantage of reducing the coronary arterial steal associated with modified Black–Taussig shunt, but it has the disadvantage of the need to perform a ventriculotomy that may affect ventricular function and trigger arrhythmias. A single ventricle reconstruction trial has been conducted since 2005 to compare m-PTS and RV-PAS by evaluating death or transplantation-free survival at 12 months after randomization. Although RV-PAS showed a better primary outcome at 12 months, the Norwood procedure with RV-PAS had no significant transplantation-free survival at 3-year analysis compared with modified Black–Taussig shunt. In addition, 19 patients underwent cardiac transplantation during the 3-year observation period of this trial and the associated independent risk factors were verified by multivariate analysis. The interim results showed that ventricular function before the Norwood procedure, genetic syndrome, and non-HLHS diagnosis, rather than the shunt types, might be risk factors for transplantation.

A hybrid surgical procedure was developed as a less invasive approach, which was initially performed by stenting of the arterial duct combined with bilateral pulmonary artery banding and arterial septostomy to achieve an alternative Norwood procedure. Although there have been many different techniques applied as a hybrid approach to palliate neonates with single ventricle lesions, these 3 major surgical palliation strategies showed comparable clinical outcomes during stage 2 (bidirectional cavopulmonary shunt) and stage 3 palliations (total cavopulmonary shunt) by direct comparison analysis. Frequent repeated intervention might be required in patients who underwent either RV-PAS or the hybrid approach compared with modified Black–Taussig shunt; however, ventricular dysfunction after palliation, in all strategies,
was substantiated as a critical risk factor that may be associated with late adverse events including death or transplantation.

Late Outcomes in Functional Single Ventricle
Cardiac death related to CHD has decreased dramatically over the past decades, but further challenges remain in patients with complex heart malformations. In single ventricle lesions, a stage 3 palliation, the Fontan procedure, may substantially improve systemic oxygen saturation; however, the absence of a subpulmonic pump may be insufficient to produce passive anterograde blood flow through the pulmonary vasculature, resulting in elevated ventricular filling pressure and high pulmonary vascular resistance. Although long-term survival after the Fontan procedure has improved in recent decades, multiple long-term complications, including protein-losing enteropathy, chronic congestive hepatopathy, thrombosis, atrioventricular valve regurgitation, and tachyarrhythmias, associated with Fontan physiology may exacerbate ventricular dysfunction. Although a surgical approach, such as the creation of a fenestration between the systemic venous conduit and the pulmonary venous atrium, is anticipated to reduce ventricular filling pressure, strategies to preserve ventricular function after staged palliation remain a critical challenge.

Heart Transplantation in Patients With Single Ventricle Lesions
Heart transplantation remains the final end-stage option to treat patients with CHD; however, the less frequent use of mechanical circulatory support in patients with CHD, because of the higher risk of mortality after implantation, has led to a lower urgency status and resulted in less likelihood for the need for a transplant after listing compared with patients without CHD. Indeed, the number of infants who received heart transplants has decreased, which may be because of limited donor availability for an increasing proportion of pediatric transplants, but is also caused largely by recent advances in surgical palliation to treat infants with single ventricle lesions. An emerging option to treat neonates with end-stage heart failure is the use of a hybrid procedure to stabilize ductus-dependent circulation in critical newborns for bridging to transplantation or a delayed Norwood procedure.

Neonatal heart transplantation is uncommon and remains as an option only in selected centers. Primary heart transplantation in neonates with single ventricle physiology, in particular those at high risk of stage I surgical palliation, was thought to be beneficial by reconstructing biventricular circulation. There has been no randomized study to compare the clinical outcomes between such high-risk patients selected for primary transplantation versus Norwood palliation. Moreover, patients with a failed Norwood procedure undergoing rescue transplantation have shown significantly higher mortality compared with those that received the transplants as a primary treatment. Future studies are anticipated to investigate the hybrid procedure as an alternative approach to replace Norwood palliation followed by heart transplantation in high-risk patients with single ventricle lesions.

An early study has shown that heart transplantation may be more applicable at stage 2 transition, rather than at shunt stage 2 or after stage 3 completion, to prevent late developing circulatory failure in certain high-risk patients. A large multicenter trial also followed up this study and showed that patients with single ventricle physiology who received the Fontan procedure had worse survival outcome after heart transplantation compared with those with biventricular pediatric heart disease. However, recent studies have demonstrated equal clinical outcomes in patients with failed Fontan circulation, suggesting an alternative indication for transplantation. These results were controversial and need to be addressed by future studies using the same criteria for transplantation.

Medical and Device Therapies for Heart Failure With Single Ventricle Physiology
The initial phase of clinical studies to treat CHD patients with symptomatic heart failure focused on 2 major pharmacological agents such as β-blockade and angiotensin-converting enzyme inhibition. The first randomized-controlled study showed that β-blockers had no significant impact on improvement in clinical heart failure outcomes in children and adolescents with CHD, but indicated a possible differential effect of carvedilol on systemic left heart morphology. A retrospective study found that carvedilol combined with standard therapy might be effective in patients with completion of the Fontan procedure rather than stage 2 palliation. The involvement in ventricular morphology to predict the therapeutic response could not be elucidated because of the heterogenic and small study population. Pharmacological blockade of the renin–angiotensin system in infants with single ventricle physiology and in adults with systemic right ventricle has been reported by 2 independent, multicenter, randomized trials, and showed that losartan improved neither cardiac function nor heart failure status, suggesting a minimal increase in the renin–angiotensin system in the population with heart failure.

In contrast, there is growing interest to elucidate whether pulmonary vasodilators may reduce pulmonary vascular resistance, resulting in ventricular preload reduction. Several reports have shown that sildenafil treatment in patients with Fontan circulation may improve exercise tolerance and oxygen consumption at the anaerobic threshold. A more recent randomized trial by using the endothelin-1 receptor antagonist, bosentan, also supported the idea that pulmonary vasoconstrictor blockade may be effective to improve exercise capacity in patients with Fontan circulation.

Cardiac resynchronization therapy may be effective in a subset of adult heart disease patients; however, the use of cardiac resynchronization therapy in pediatric patients has been limited because the complex anatomic substrate of CHD and myocardial scar formation secondary to repeated surgery may hinder the traditional pacing approach to restore effective synchrony. Pediatric patients with dilated cardiomyopathy (DCM) seemed to be nonresponsive to cardiac resynchronization therapy, which is in contrast to the favorable results seen in adults. Patients with repaired tetralogy of Fallot may require an atrial synchronous single-site pacing to avoid the opposite direction of the activation wavefront from spontaneously occurring wave fronts. With respect to single ventricle lesions, resynchronization must be achieved by multisite
pacing because of the deformation of ventricular morphology by chronic pressure and volume overloads. Only a few studies in a small number of patients have evaluated the effect of cardiac resynchronization therapy in single ventricle physiology; however, the results are inconsistent and need further investigation.56,57 This uncertainty could be explained, in part, by the dissected relationship between electric- and mechanical-dysynchrony found in patients with HLHS.58

Although a variety of ventricular assist devices have been used as a bridge to transplantation in patients with failing systemic right ventricles, studies in patients with single ventricle physiology remain more challenging.59,60 The Berlin Heart EXCOR experience has shown that a functional single ventricle was found to be at high risk of wait-list and posttransplant mortality relative to 2-ventricle patients with CHD; however, there is some evidence to show that the use of an EXCOR pediatric ventricular assist device provides satisfactory results for the bridge to transplantation in patients with stage 2 or stage 3 palliation in single ventricle physiology.61

**Preclinical Studies of Cell Therapy Relevant to CHD**

In addition to the aforementioned advances in a variety of surgical and medical care options in patients with CHD, substantial myocardial regeneration has recently emerged as a novel therapeutic strategy to treat children with heart failure.62 For preclinical proof-of-concept studies in CHD, several cell types have been used in pressure–overload right heart models. An initial report of direct myoblast transplantation in an ovine model did not show a functional benefit, with few cells integrating on the right myocardium.63 The development of sheet technology has enabled an increase in cell engraftment, resulting in increased ventricular function and reduced cardiac fibrosis with enhanced vasculogenesis.64 The second wave of recent advances may be the intramyocardial delivery of umbilical cord blood stem cells in large animal models. The safety and feasibility of this procedure were confirmed; however, the observed functional benefits could not be explained by substantial cardiomyocyte differentiation rather than the presence of hematopoietic cells on the transplanted myocardium.65,66

C-KIT–expressing CPCs have been extensively studied in heart disease because of the advantage of their natural location, their function in the heart to preserve homeostasis during the aging process, and their support of cardiac function, as proven by their beneficial effects resulting from potential myocardial-lineage differentiation after transplantation.67 These cells are clonogenic and multipotent to give rise to cardiomyocytes, smooth muscle cells, and endothelial cells in vitro and contribute to de novo formation of myocardium and vessels in vivo.10 However, recent studies with genetically lineage-tracing approaches have reported that c-kit–expressing CPCs from the adult mouse heart have not been shown to differentiate into cardiomyocytes in vitro or transplantation into injured hearts.68,69 Following studies suggest that c-kit–expressing CPCs may contribute to endothelial cell production rather than functionally sufficient myocyte generation in the infarcted hearts.70,71

Another population with CPC that has been intensively investigated in preclinical and clinical studies may be CDCs. Human CDCs and their derivatives have been shown as clonogenic and endogenous heart in origin, and could give rise to cardiomyocytes in vitro and in vivo.72–74 Importantly, paracrine effectors secretion through the transplanted CDCs after infarction contributed to the functionally meaningful renewal and repair in the injured hearts, and majority of the functional CDCs were considered as non–c-KIT-expressing cell population.75,76 On the basis of preclinical proof-of-concept studies in these two types of human CPCs,77–79 phase 1 clinical trials of autologous CPCs have been reported in patients with recent myocardial infarction to test the feasibility and the procedural safety.80–82 The CDC-based cell therapy for cardiac repair was currently implemented in adult patients with DCM by allogeneic transplantation strategy.83

The results of intracoronary administration of CDCs in an animal model of right heart failure were encouraging. As shown in Figure, we investigated the contribution of CDC infusion to pulmonary hypertension-associated right ventricular failure in a rat model of pressure overload. Four weeks after pulmonary artery banding, right ventricular hypertrophy and interstitial fibrosis were noted (Figure B, E, and H). On intracoronary administration of rat CDCs, cardiac fibrosis decreased remarkably, but the myocyte diameter in the right ventricle remained unchanged 4 weeks after cell transplantation (Figure C, F, and I). Dose-escalation study demonstrated that 1×10⁷ cells of rat CDC infusion could efficiently reduce the fibrotic area but myocyte hypertrophy remained (Figure J and K). The histological findings were confirmed by the obvious engraftment of injected rat CDCs within the endocardium in right ventricle and perivascular lesions 4 weeks after CDC infusion (Figure L). A readily detectable engraftment was observed in the fibrotic area (Figure N: 4.4%±0.3%: X-gal–positive cells per total cells counted). Quantitative analysis showed that a significantly greater number of the engrafted CDCs, those were identified by β-galactosidase–(LacZ)positive cells, could be appreciated as α-sarcomeric actin-expressing cardiomyocytes in the fibrotic area compared with those in nonfibrillar lesions (Figures M and O; 33.8%±5.6% versus 18.1±2.1%, P=0.043). These results provided the rationale of CDC dose (3.0×10⁷/kg of body weight) that we clinically applied in patients and the evidence that cardiac functional improvement might be, at least in part, the results of substantial myocyte regeneration and reduction of cardiac fibrosis followed by CDC infusion in a right heart failure model.

Mesenchymal stem cells (MSCs) are mesoderm-derived stem cells that can be isolated from various organs, including bone marrow, umbilical cord blood, and adipose tissue.84 Human MSCs were used as a therapeutic strategy to treat a porcine model of pressure overload in the neonatal right ventricle.85 Intramyocardial delivery of human MSCs has been shown to preserve global and regional cardiac functions, attenuate remodeling, and stimulate endogenous progenitor cell proliferation to promote neovascular formation and myocyte cycling. It should be noted that right ventricular hypertrophy could be reversed via growth differentiation factor 15 signaling directly secreted through MSCs.
Figure. Preclinical study of intracoronary cardiosphere-derived cell (CDC) infusion in a rat model of right heart failure. A–K, Pulmonary artery (PA) banding was created to induce pressure overload right heart failure in rats (weighing 250–300 g). The left thorax was opened to expose the pulmonary artery. A silk suture was tied tightly around an 18-gauge needle alongside the pulmonary artery, followed by a rapid removal of the needle to leave the pulmonary artery constricted in the lumen equal to the diameter of the needle. Intracoronary infusion was performed 4 weeks after pulmonary artery banding. The ascending aorta and the pulmonary artery were occluded with a snare twice for a 20-s interval, 10 min apart, during which time rats received an infusion of CDCs or vehicle into the aortic root directly. Animals were euthanized at 4 weeks after treatment to obtain immuno-histological data. Masson-trichrome staining and hematoxylin and eosin (H&E) staining are shown. One month after pulmonary artery banding, significant right ventricular (Continued)
Figure Continued. Hypertrophy and fibrosis were observed. Note that CDC treatment reduced cardiac fibrosis, but not hypertrophy, one month after infusion (F and I). Bars, 2 mm in A to C; 20 μm in D to I. J, Animals were separated into 4 groups: (1) sham-operated animals (n=12), (2) rats subjected to pulmonary artery banding for 4 weeks with vehicle treatment (n=12), and (3 and 4) rats subjected to banding for 4 weeks with 2 doses of CDC infusion (0.5×10^5 or 1×10^5 cells: n=12 in each). Cardiac fibrosis induced by right ventricle pressure overload was measured. CDC infusion significantly reduced the fibrotic area in a cell dose-dependent manner. K, CDC treatment did not affect the diameter of myocytes, which are mechanically enlarged by pressure overload. L, Rat CDCs were infected by lentiviral vectors harboring human cytomegalovirus promoter-driven LacZ reporter gene and were subjected to intracoronary transfer into rats 4 weeks after pulmonary artery banding. Clear CDC engraftment could be detected along the endocardium and surrounding capillary vessels where the cells had been injected. M, Cardiomyocytes were stained with α-sarcomeric actin (red). Newly regenerated LacZ-positive cardiac muscle cells could be detected by β-galactosidase staining (green). Substantial cardiomyocyte regeneration was verified 4 weeks after CDC delivery. Bars, 50 μm. N, Engrafted LacZ-positive CDCs were evaluated by X-gal staining and corrected by the number of total nuclei appreciated within the respective area. Fibrotic and nonfibrotic areas were determined by Masson-trichrome staining derived from serial sections. O, Differentiated cardiomyocytes after CDC infusion were verified by the cells coexpressing both α-sarcomeric actin (red) and β-galactosidase (LacZ, green). The frequency of the cells coexpressing α-sarcomeric actin among the β-galactosidase-positive cells is shown. Data are expressed as the means (SD).

With respect to preclinical studies of DCM, the therapeutic efficacy of these 4 types of progenitor cells, as described above, has been elucidated using genetically manipulated or chemically induced cardiomyopathy models in small animal experiments with encouraging results.68–92 Limited, but increasing, efforts have been made to conduct preclinical proof-of-concept studies in small and large animals, and the results have paved the way for various types of progenitor cells as potential therapies to treat patients with CHD.

Early Case Reports of Cell Therapy in CHD

Over the last years, pediatric cardiologists and cardiovascular surgeons have evaluated the safety and preliminary efficacy of umbilical cord blood stem cell and bone marrow–derived mononuclear cell transplantation by case reports in the end-stage of pediatric heart failure, such as DCM,94–96 double outlet right ventricle, pulmonary atresia with ventricular septal defect,97 HLHS,98 and myocardial infarction secondary to Takayasu arteritis.99 Although the underlying pathogenesis may differ among the diseases in children, initial results related to safety and feasibility, either by intramyocardial or transcoronary transfer, were encouraging.100 As pioneering investigations into CHD, these preliminary reports have suggested the potential of progenitor cell therapy to treat children as indicated by markedly improved cardiac function and reduced heart failure status; however, the follow-up studies have suggested a subpopulation who did not respond to the therapies and died or required subsequent heart transplantation.77,101 Larger-scale clinical trials are needed to ascertain the risk and benefit as a bridge to transplantation therapy of these progenitor cell transfers in the terminal stage of heart failure in children.

Advantage of Heart-Derived Progenitor Cells in Children

Direct comparison of CDCs between children and adults has shown a fact that there might be a greater abundance of these cells within the heart of patients with CHD.102 Similarly, c-KIT–expressing CPCs were found to be 3-fold higher in neonates than in children as aged >2 years of age.103 Neonatal c-KIT–expressing CPCs have also revealed to have higher proliferative capacity compared with those from adults.14 In addition, human CDCs in patients with CHD showed the highest expression of c-KIT and NKX2-5, a cardiac-specific transcription factor, during neonatal period and that decreased with increasing age.12 An independent study has elucidated the unique characteristics of CDCs in children. The results of in vitro and in vivo experiments have demonstrated a greater potential for myocardial regeneration and neoangiogenesis, partly conferred by the production of a series of significant trophic factors through progenitor cells of children compared with those of adults.13 These age-dependent functional characteristics of CPCs were further investigated by using c-KIT–expressing CPCs. Transplantation of neonatal CPCs into rat myocardial infarction produced significantly greater functional recovery through increased neovascular formation and paracrine factors secretion when compared with that of adult heart-derived CPCs.14 Interestingly, neonatal CPC-derived total conditioned media could recapitulate these beneficial effects but not in that of adult CPCs, suggesting neonatal CPC secretome may play an important role in these processes. Furthermore, systematic proteomic analysis has identified heat shock factor 1, a master heat shock protein regulator, may be a critical transcription factor to regulate the functional potential of the CPC-mediated secretome. This transcription factor controls the expression of heat shock protein 70, which is one of the highly expressed canonical signaling pathways in neonatal CPC-derived total conditioned media. These dynamic changes in c-KIT–expressing CPC function could be simply explained by age-dependent cellular and biological loss-of-function along with a developmental program that could be determined by transcriptional and post-translational regulatory networks and noncoding RNAs.102

In addition, an age-matched comparison between patients with CHD and end-stage CHD has shown that CPCs increased in patients with end-stage heart failure, but reduced their replicative capacity compared with those of CHD.103 These results indicated that inflammatory response and mechanical stress may alter the extracellular matrix conformation in the heart to affect resident progenitor cell signature and function. Although further studies are needed to verify specific factors or proteins that can enhance progenitor cell function, understanding the regulatory mechanisms of CPCs in children is critical to achieve an optimal result for regenerative medicine.

Cell Therapy Trials in Patients With CHD

Because of the uncertainty about the impact of progenitor cell therapy to treat patients with CHD, the TICAP (transcoronary infusion of CPCs in patients with single ventricle physiology) phase I trial was undertaken starting in 2011 as
the first complete clinical study in children with a diagnosis of HLHS.104 The TICAP trial was designed to prospectively enroll 7 patients receiving CDC infusion 1 month after stage 2 or stage 3 palliation and to compare them with 7 patients treated by a standard surgical procedure alone without placebo infusion. The primary outcome was to evaluate the procedural feasibility and safety of intracoronary infusion of CDCs after staged palliation. Procedural complications were evaluated by distal coronary embolism, acute coronary injury, and sustained ventricular arrhythmia associated with CDC infusion. Secondary outcome measures included the incidence of hospitalization for heart failure, ventricular arrhythmia, myocardial infarction, general infection, and renal and hepatic dysfunction followed by CDC infusion. As a preliminary efficacy evaluation, cardiac function was analyzed at 3 to 18 months after protocol treatment by 3 different imaging modalities and compared with the baseline.

The TICAP trial enrolled 18 eligible participants, of which 14 patients completed the staged surgical palliation and allocated to protocol treatment. No serious adverse events associated with CDC infusion were observed during the early to late phase of follow-up. The preliminary efficacy of CDC infusion was validated as a significant increase in ventricular function and reduction in tricuspid valve diameter, as well as ventricular volumes measured by cardiac MRI, echocardiography, and ventriculogram. These beneficial effects had an impact on improved heart failure status and somatic growth with significantly less unplanned catheter intervention in CDC-treated patients compared with controls after staged procedures. The 3-year follow-up results of the TICAP study confirmed these safety issues and no ectopic tumor formation, associated with CDC infusion in the long-term. The favorable effects remained in CDC-treated patients,105 whereas the control patients showed no significant changes in ventricular function, which is consistent with the results reported by interstage analysis of a single ventricle reconstruction trial and a cross-sectional study in Fontan survivors.26,36

These initial results of the TICAP phase 1 study have been carefully validated by the PERSEUS (CPC infusion to treat univentricular heart disease) phase 2 trial.106 This phase 2 trial consists of a randomized, controlled study enrolling 34 participants and comparing the therapeutic efficacy of CDC after stage 2 or stage 3 palliation in a wide range of patients with single ventricle physiology, including previously reported subjects with HLHS. The primary end point was to assess ventricular function at 3 months compared with baseline. The control subjects may have a late CDC infusion, at least 4 months after the staged surgery, as an option when the primary end point analysis was completed. Secondary outcome measures included cardiac function improvement at 12 months relative to baseline and associated heart failure status measurement.

In this larger phase 2 randomized study, the therapeutic efficacy of CDC infusion was confirmed at 3 months of follow-up evaluation. Similar to the results of TICAP phase 1 study, CDCs favorably affected the cardiac function in primary- and late-CDC transplantation. These beneficial effects were not restricted to the types of shunt palliation that patients received before the cell infusion; however, the therapeutic responsiveness was apparently correlated with the cardiac function before the treatment. As part of the mechanistic insights, CDC-induced functional benefits could be appreciated by reduced myocardial fibrosis that may lead to improved ventricular stiffness by reverse remodeling. Trophic effectors secretion from the transplanted subjects, but not in controls, was simultaneously monitored and 2 factors, such as insulin-like growth factor-1 and hepatocyte growth factor, were identified as candidates that may be involved in the process of myocardial regeneration. In addition, the clinical outcomes at 12 months of follow-up revealed a significant reduction in heart failure status and in the levels of bone morphogenetic protein. The cardiac function improvement found at 3 months was retained to a similar degree at 12 months. Most importantly, CDC-treated patients showed improvement in health-related quality of life at 12-month observation, and this may greatly affect the relief from parenting stress of their families as well.

Although there have been only these 2 complete cell therapy trials reported to assess the safety and efficacy in patients with CHD, several clinical studies are currently being initiated to pursue the initial results of the TICAP and PERSEUS studies. The APOLLON (cardiac stem/progenitor cell infusion in univentricular physiology: NCT02781922) phase 3 trial, which is a successor of the TICAP phase 1 and PERSEUS phase 2 studies, was implemented by a sponsor-initiated multicenter trial in 3 children’s hospitals to compare the therapeutic efficacy between controls and CDC-treated subjects for 12 months by enrolling 40 participants with single ventricle physiology (Table). There are several ongoing clinical trials to determine the procedural safety and preliminary efficacy in patients with HLHS, DCM, and Duchenne muscular dystrophy by adopting a variety of cell types and delivery approaches (Table).107 Among the studies, 2 trials were conducted by allogeneic cell transplantation based on the preclinical and clinical proof-of-concept investigations.108,109 These ongoing clinical trials are in response to the severe unmet medical need for novel regenerative strategies in the field of CHD.

Mechanisms of Stem Cell–Based Cardiac Repair in Children

There are many uncertainties related to stem cell therapies to treat patients with CHD and adult heart disease. In short-term observations, it was previously believed that transplanted cells could give rise to functional cardiomyocytes, endothelial cells, and smooth muscle cells to create new cardiac tissue in an ischemic myocardial environment.77–79 However, further investigation has shown that the engraftment rate of transplanted cells was low in long-term, suggesting a likelihood of insufficient number of differentiated cardiovascular cells to directly replenish the damaged tissue to produce measureable improvement in cardiac function.110 In addition, the lineage-tracing experiments have revealed that vasculogenesis rather than cardiomyogenesis, followed by transplantation of either bone marrow-derived mononuclear cells or resident heart-derived progenitor cells, might be an alternative mechanism of action that could be critically involved in the processes of myocardial regeneration.111,112
The prevailing hypothesis is that transplanted cells may create a local milieu and stimulate endogenous cell recruitment and proliferation for regeneration by secreting paracrine cardioprotective factors, as previously profiled and compared among the cell types.11 These trophic factors may stimulate innate progenitor cell activation to promote vascular growth and cardiomyocyte differentiation, resulting in attenuation of fibrosis and modulation of inflammatory responses.113 Early studies have shown that factors constituting this regeneration process may involve specific cytokines, angiogenic growth factors, and proper factors for stem cell mobilization or recruitment.114 Recent studies have revealed that these factors may be clustered into extracellular membrane vesicles such as exosomes. Exosomes are lipid bilayer nanovesicles secreted by a variety of the cells when multivesicular endosomes fuse with the plasma membrane.115 It is notable that injection of c-KIT-expressing neonatal CPC-derived exosomes improved cardiac function in experimental myocardial infarction, whereas those from older children did not show any beneficial effects.117 In addition, hypoxic stimuli could restore the defective reparative potential in older patient–derived exosomes and significantly reduced cardiac fibrosis. These results suggest that the potential advantage of CPCs in patient with CHD might be regulated by oxygen levels and age of donor cells. Pediatric CPC-derived exosomes and the packed microRNA could be the mediators involved in the intracellular communication between transplanted CPCs and host myocardium. Although the CPC-derived exosomes-mediated cardiac repair in CHD is under study, the functional benefits observed in vivo by cell-secreted exosomal microRNA may be age-dependent of autonomous cells and hold the possibility to substitute cell-based regeneration therapy.117,118

The most noticeable differences in the pathogenesis of heart failure between patients with CHD and ischemic cardiomyopathy might be the presence of atherosclerotic lesions in adult patients with ischemic heart disease, but the absence of such lesions in children with CHD. This fundamental difference could be attributed to the extent of myocardial damage and might be associated with adverse cardiovascular events including ventricular dysfunction. Alternatively, the difference between these 2 heart diseases may be explained by the extent of myocardial fibrosis, which is an import determinant of the development and progression of heart failure.119 In the diseased heart, injured cardiomyocytes are lost to cell death, activating innate immune signals to trigger intense inflammatory responses. During the myocardial healing processes, suppression of inflammatory responses is associated with endothelial cells and activation of myofibroblasts to initiate the reparative fibrosis.120 Formation of scar tissue in the damaged myocardium plays an important role in the preservation of myocardial integrity; however, a hyper-reactive reparative program has been proposed to cause pathological ventricular remodeling. Late gadolinium enhancement by cardiac MRI has become an available tool to detect myocardial fibrosis and infarction. Patients with ischemic cardiomyopathy invariably show late gadolinium enhancement as areas of scarred myocardium, whereas patients with single ventricle physiology who had Fontan completion and those with DCM are reported to show cardiac fibrosis at 28% and 16%, respectively.121,122 These findings imply a limited role of myocardial fibrosis involved in the pathogenesis of CHD. Although children with CHD have a similar degree of practical ventricular dysfunction as that observed in adults with ischemic cardiomyopathy, the absence or lesser degree of myocardial fibrosis seen in children may physiologically indicate a viable but dysfunctional myocardium as unique structural changes in CHD that contributes to distinct reparative mechanisms of cell therapies in children.123 Recent advances in T1-mapping technology may enable us to discern the diffuse myocardial disease and quantitate the extracellular matrix content for understanding the basis of myocardial adaptations to stem cell therapies in CHD.124

Increased hemodynamic workload in CHD may produce substantial myocyte loss by a disruption of intercellular communication as well as structural and functional integrity of cardiovascular cells, resulting in heart failure and arrhythmias associated with cardiac death. Patients with complex CHD have also shown to have microvascular dysfunction such as abnormalities include reduced capillary density and impaired myocardial blood flow reserve as a defective vascularization.125 Recent preclinical studies have revealed that angiogenic factors secreted by MSCs may increase capillary density in parallel with a subset of transdifferentiation of pulmonary vascular endothelial cells by transplanted cells in a model of pulmonary hypertension.126-128

Children with CHD are invariably exposed to chronic hypoxia with pressure and volume overload. Hypoxia-inducible factors (HIFs) are α/β heterodimeric transcription factors binding to hypoxia response elements in response to oxygen-sensing dioxygenases.129 Fetal heart development occurs in a hypoxic condition, and activation of the HIF system directly

Table. Ongoing Cell Therapy Trials to Treat CHD

<table>
<thead>
<tr>
<th>Trial Name</th>
<th>Disease</th>
<th>Trial Design</th>
<th>Sample Size</th>
<th>Cell Used</th>
<th>Cell Type</th>
<th>Delivery</th>
<th>Follow-Up</th>
<th>Phase</th>
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</tr>
</thead>
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<tr>
<td>APOLLON</td>
<td>SVP</td>
<td>Randomized</td>
<td>40</td>
<td>Autologous</td>
<td>CDCs</td>
<td>IC</td>
<td>12 mo</td>
<td>3</td>
<td>NCT02781922</td>
</tr>
<tr>
<td>ELPIS</td>
<td>HLHS</td>
<td>Randomized</td>
<td>30</td>
<td>Allogeneic</td>
<td>BM-MSCs</td>
<td>IM</td>
<td>12 mo</td>
<td>1</td>
<td>NCT02398604</td>
</tr>
<tr>
<td>–</td>
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<td>Single Arm</td>
<td>10</td>
<td>Autologous</td>
<td>UCBcs</td>
<td>IM</td>
<td>24 mo</td>
<td>1</td>
<td>NCT01883076</td>
</tr>
<tr>
<td>–</td>
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<td>Randomized</td>
<td>32</td>
<td>Autologous</td>
<td>BM-MNCs</td>
<td>IC</td>
<td>3 mo</td>
<td>1/2</td>
<td>NCT02256501</td>
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<tr>
<td>HOPE</td>
<td>DMD</td>
<td>Randomized</td>
<td>24</td>
<td>Autologous</td>
<td>CDCs</td>
<td>IC</td>
<td>12 mo</td>
<td>1/2</td>
<td>NCT02485938</td>
</tr>
</tbody>
</table>

BM-MSCs indicates bone marrow–derived mesenchymal stem cells; CDCs, cardiosphere-derived cells; CHD, congenital heart disease; DCM, dilated cardiomyopathy; DMD, Duchenne muscular dystrophy; ELPIS, Allogeneic hMSC Injection in Patients With Hypoplastic Left Heart Syndrome; HOPE, Halt Cardiomyopathy Progression in Duchenne; HLHS, hypoplastic left heart syndrome; IC, intracoronary; IM, intramyocardial; NCT ID, ClinicalTrials.gov Identifier; SVP, single ventricle physiology; and UCBcs, umbilical cord blood cells.
contributes to normal cardiac morphogenesis.\textsuperscript{130,131} The results of overexpression of HIF-1α in the postnatal heart are controversial and have been associated with improved cardiac function after infarction but development of heart failure with spontaneous cardiomyopathy in other studies.\textsuperscript{132,133} In theory, activation of HIF pathways can provide protection against myocardial damage by multiple mechanisms such as induction of angiogenesis by ischemic preconditioning. Several animal studies have shown favorable effects of the HIF system through a preconditioning stimulus that can be operated directly, remotely, or by both.\textsuperscript{129,134} Although hypoxia is also a major component of ischemic cardiomyopathy, this preconditioning is invariably seen in children with CHD, whereas not in all adult heart disease patients. Structural heart disease, in particular, may show a differential disease progression, as observed by the lesser extent of focal cardiac fibrosis relative to ischemic cardiomyopathy. The principle behind the therapeutic modulation of the HIF system was further investigated by priming stem cells to have an enhanced protective effect under hypoxic circumstances.\textsuperscript{117,135,136} In children with single ventricle physiology after stage 2 palliation, the myocardium under hypoxic conditions remains as a postconditioning milieu that may confer additional myocardial protection mechanisms indirectly through the transplanted cells.

### Strategies for Cell Delivery

The most common cell delivery system used in patients with CHD is the direct injection of the manufactured cells through coronary arteries. Intramyocardial injection or cardiac patch implantation during open chest surgery may be the alternatives. Direct myocardial injection may be limited by the intracellular space available for delivering high dose of cell suspension. Cardiac patch technologies have been investigated to improve cell engraftment and survival for optimal therapeutic benefit; however, the functional integration such as immediate electric-coupling with host myocardium remains to be elucidated.\textsuperscript{137} Engineered cardiac patches by scaffold have enabled to release paracrine factors or bioactive peptides into targeted myocardium in a controlled manner. Intravenous cell injection into biventricular heart disease has been considered as inefficient because of the low cell retention and rapid diffusion to other organs during circulation; however, children with single ventricle physiology before stage 3 palliation may be applicable to this delivery approach because the venous return from inferior vena cava may directly supply into functional single ventricle and related coronary arteries. Appropriate cell-dose and age for injection could be additional questions needed to be addressed by further preclinical and clinical studies. The high priority of cell therapy in patients with a variety of CHD remains elusive.

### Future Directions

Small and long noncoding RNAs have been shown to negatively regulate gene expression and signaling pathways in various tissues. Studies have provided new insight into the mechanisms of how tissue noncoding RNA expression contributes to the control of myocyte and vessel growth, contractility, and myocardial fibrosis.\textsuperscript{138-140} More recently, noncoding RNAs have been implicated in the pathogenesis of several types of CHD and circulating noncoding RNAs may be attractive clinical biomarkers for disease diagnosis.\textsuperscript{141,142} Whole-genome microRNA screening has shown that a subset of microRNAs stimulate endogenous cardiomyocytes to proliferate and promote myocardial regeneration.\textsuperscript{143} Furthermore, animal studies have shown that inhibition of microRNA-208a and microRNA-21 may reduce cardiac fibrosis to prevent myocardial remodeling.\textsuperscript{144-146} Although the therapeutic potential of noncoding RNAs in CHD has not yet been reported, cross-sectional analysis between microRNAs and CPC-derived exosomes from children and adolescents has indicated that microRNA-mediated signaling may be age-specific and can provide critical information leading to novel therapeutic strategies in CHD.\textsuperscript{117}

Increasing studies have used induced-pluripotent stem cells to repopulate the injured myocardium or recapitulate the disease phenotype in humans to identify novel therapies.\textsuperscript{147-149} Therapeutic potential of exosomes derived from induced-pluripotent stem cells and somatic stem cells may confer a cardioprotective preconditioning effect in injured myocardium.\textsuperscript{150} Direct reprogramming of cardiac fibroblasts toward functional cardiomyocytes by defined transcription factors or small molecules may substitute this technology by overriding the complex manufacturing processes in clinical practice.\textsuperscript{151-153} Cardiomyocyte proliferation has been achieved by deletion of transcription factor Meis1, as well as by administration of recombinant protein neuregulin-1 in mice\textsuperscript{157,154}; however, few clinical trials have been reported in patients with heart failure, and challenges remain for research on mechanisms and preclinical studies related to CHD.\textsuperscript{155}

In light of recent advances in cardiac regeneration therapies in adult heart disease, there are still many questions that remain unanswered with regard to the differential and proper physiology and pathophysiology mechanisms occurring during reparative processes in CHD. The pooled results from preclinical and clinical studies may require comprehensive meta-analysis to verify the therapeutic efficacy.\textsuperscript{156-158} Most importantly, characterization of treatment responders in clinical studies may provide a highly specific foundation for the next generation of therapies for patients with CHD.\textsuperscript{159}

### Conclusions

Patients with CHD, including single ventricle physiology, who survive after surgical procedures require lifelong medical attention and may have late-onset complications, such as advanced heart failure, that can place an enormous stress on families. Recent preclinical and clinical studies of adult heart regeneration have revealed that transplantation of stem cells into the heart was a feasible and safe method to treat patients with heart failure. Although the functional benefits of cell therapy might be largely mediated by autocrine or paracrine factor secretion to repair the injured myocardium rather than de novo myocardial differentiation in situ, the achieved consensus has shed light on proteins and noncoding RNAs transferred by stem cell–secreted exosomes to mediate the protective effects. The clinical efficacy of stem cell therapies in adult heart disease remains elusive, and the mechanisms underlying the regenerative processes in children are still unclear. Cell therapy trials for CHD have just been initiated.
The advantage of the biological significance of resident CPCs from CHD should be taken into account for disease modeling and development of novel regenerative therapies. New insights into CPCs and MSCs isolated from pediatric patients, as well as molecular mechanisms controlling the cell proliferation and functional differentiation of these cells during stressed workload, may provide the foundation for exciting regenerative approaches for CHD treatments.

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Disclosures

None.

References


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