Cardiac stem cell therapies for congenital heart diseases

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During the last 2 decades, stem cell therapies with bone marrow mononuclear cells (BMMNCs) or mesenchymal stem cells (MSCs) to treat ischemic heart disease, including in pre-clinical and clinical trials, have demonstrated the ability of stem cells to improve cardiac function, infarct size, and cardiac remodeling in adult patients. In recent years, endogenous cardiac stem cells (CSCs) derived from heart tissue have been identified. CSCs have been shown to have superior regenerative potential over other types of stem cells in terms of cardiovascular-lineage differentiation, paracrine factor secretion, and functional improvement after cell transplantation. Cardiac stem cell therapy to regenerate damaged myocardium after chronic infarction has been reported in the SCIPIO and CADUCEUS trials. In contrast, although recent advances in pediatric cardiology, congenital cardiac surgery, and intensive care management have dramatically changed clinical outcomes, there is an increasing recognition of limited therapeutic improvement in children with severe heart failure. Congenital heart failure is a structural heart disease caused by multiple etiologies related to pressure and volume overload, arrhythmia, and myocardial degradation. Stem cell-based strategies to treat heart failure in adults have been investigated; however, little is known about their safety and efficacy in children and planned clinical studies are quite limited. Only case reports have been published and no large clinical trials have been conducted using any type of stem cells. Recently, the TICAP trial has revealed the safety and feasibility of intracoronary infusion of autologous cardiosphere-derived cells (CDCs) in children with hypoplastic left heart syndrome (HLHS). Although this trial had several limitations that required further evaluation, the results from this study provided a foothold for stem cell-based therapeutic strategies in patients with congenital heart disease. Eventually, a new paradigm of stem cell therapy to treat congenital heart failure has started to form. Many important issues including long-term cell engraftment, the mechanism of stem cell recruitment and differentiation, administration route, and appropriate cell types to deliver in situ remain to be investigated. Here, we review the latest research on stem cell therapies for heart failure and discuss the future perspectives on cell-based regenerative strategies to treat patients with congenital heart diseases.

Keywords: cardiac stem cells; cell therapy; congenital heart failure; intracoronary delivery

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Introduction

In the last decade, more than 5,000 patients worldwide have received some kind of cell-based therapy for a variety of cardiovascular diseases [1]. Accumulated evidence on cell-based therapy suggests its safety and effectiveness for adult ischemic heart disease [2]. However, there is also the issue of cardiac insufficiency in the pediatric population. Surgical management of congenital heart diseases including hypoplastic left heart syndrome (HLHS) has dramatically
changed clinical outcomes. Meanwhile, progressive late-onset heart failure is a serious problem, which has been increasingly recognized in children after staged cardiac surgeries [3]. The development of a new therapy to improve cardiac function for children with heart failure has been strongly desired. Theoretically, both the heart and cardiac stem cells (CSCs) of young children have a higher regenerative capacity than those of adults [4, 5], so cardiac regeneration therapy for children could be considered as a reasonable approach. However, in the clinical setting, the vast majority of clinical trials have focused on adult heart diseases rather than congenital heart diseases.

Cell types for myocardial regeneration

Sources of non-embryonic stem cells are located in the bone marrow, peripheral blood, adipose tissue, skeletal muscle, umbilical cord blood, and cardiac tissue. As shown in Table 1, various types of stem cells have been used for cardiac regenerative therapy. The characteristics of these stem cells are that they are isolated from patients for autologous transplantation with no risk of immunological rejection. We briefly summarize the types of stem cells and discuss the use of these cells for clinical trials.

<table>
<thead>
<tr>
<th>Type of stem cells</th>
<th>Origin</th>
<th>Characteristics</th>
<th>Research stage</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>skeletal myoblasts</td>
<td>muscles</td>
<td>unipotent</td>
<td>clinical</td>
<td>easy to harvest, cell culture expansion</td>
<td>low functional integration between cardiac muscles</td>
</tr>
<tr>
<td>bone marrow mononuclear cells</td>
<td>bone marrow</td>
<td>multipotent</td>
<td>clinical</td>
<td>easy to harvest</td>
<td>no specific cardiac markers</td>
</tr>
<tr>
<td>mesenchymal stem cells</td>
<td>bone marrow, umbilical cord</td>
<td>multipotent</td>
<td>clinical</td>
<td>easy to harvest</td>
<td>no specific cardiac markers</td>
</tr>
<tr>
<td>peripheral blood-derived stem cells</td>
<td>blood</td>
<td>multipotent</td>
<td>clinical</td>
<td>easy to harvest from peripheral blood</td>
<td>low number of cells</td>
</tr>
<tr>
<td>adipose-derived stem cells</td>
<td>adipose</td>
<td>multipotent</td>
<td>clinical</td>
<td>easy to harvest, cell culture expansion</td>
<td>moderate number of cells</td>
</tr>
<tr>
<td>resident cardiac stem cells</td>
<td>cardiac</td>
<td>multipotent</td>
<td>clinical</td>
<td>cell culture expansion</td>
<td>need for cardiac biopsy</td>
</tr>
</tbody>
</table>

Table 1. Cell types of stem cells for cardiac regeneration

**Skeletal myoblasts**

Efforts in the field of stem cell transplantation for heart failure were initiated with skeletal myoblasts. Skeletal muscle contains immature satellite cells, myoblasts, which have the ability to repair the muscles after acute insult. These satellite cells undergo proliferation and promote regeneration by differentiating into myotubes and new muscle fibers [6]. The advantages of skeletal myoblasts as a stem cell source are their autologous origin and the ability to expand rapidly in culture. The differentiation of undifferentiated myoblasts into cardiac-like cells has been demonstrated in canine studies [7], and pre-clinical studies showed that skeletal muscle transplantation improves cardiac function after myocardial infarction (MI) [8, 9]. In 2001, the first case of myoblast transplantation in a man aged 72 years with MI was conducted [10]. The earliest clinical studies carried out in small numbers of patients using autologous skeletal myoblasts demonstrated significant improvements in cardiac function [11]. Later, the multicenter, randomized, placebo-controlled, double-blind, phase 2 MAGIC trial was performed, which was the most comprehensive clinical evaluation of skeletal myoblasts as a cell source [12]. The results of this trial demonstrated that neither regional nor global left ventricular function assessed by echocardiography significantly improved. In addition, there was a trend towards more arrhythmias in patients treated with skeletal myoblasts. Pro-arrhythmia after skeletal myoblast transplantation may be attributable to the loss of connexin-43 expression, which results in the failure of electrical integration with the host myocardium [13]. These studies indicate that skeletal myoblasts might not be the optimal cell source for cardiac regeneration.

**Bone marrow-derived stem cells (BMSCs)**

Bone marrow-derived stem cells are the most broadly investigated for regenerative potential since their discovery in the 1960s [14]. The bone marrow contains different types of hematopoietic and non-hematopoietic stem cells. These stem cells constitute up to 2% of the total bone marrow and have the ability to differentiate into diverse phenotypes. Similar to skeletal myoblasts, these cells are easy to harvest and culture. BMSCs have been used in most of the preclinical and clinical trials for cardiac regeneration.

**Bone marrow mononuclear cells (BMMNCs)**

Bone marrow mononuclear cells (BMMNCs) can be easily procured by density gradient centrifugation and these cells do not need a difficult culture technique. For these reasons, they have been widely used in animal experiments. In the early 2000s, BMMNCs were shown to have the potential to undergo myogenic differentiation in mice [15, 16]. Another group demonstrated that BMMNCs secreted potent angiogenic cytokines such as vascular endothelial growth factor and induced the proliferation of BM-derived as well as
endogenous vascular and endothelial cells in pigs with chronic MI [17]. These findings on the cardiac regenerative potential of BMMNCs promoted clinical studies for human cardiac repair.

In 2002, the first clinical trial of intracoronary infusion of autologous BMMNCs in patients with acute MI was carried out [18]. Following this trial, several clinical trials using BMMNCs, including TOPCARE-AMI [19], the BOOST trial [20], REPAIR-AMI [21], TIME [22], and Late-TIME [23], were conducted, but the benefits of BMMNCs remained controversial. To avoid the potential bias associated with a small number of participants in these clinical trials, meta-analyses of human cardiac regenerative therapies have also been reported. In 2012, Jeevanantham et al. performed a systematic review and meta-analysis of pooled data from 50 trials, enrolling 2,625 patients, to assess BMMNCs [2]. They reported significant improvements of left ventricular (LV) function, infarct size, and remodeling in patients with ischemic heart disease after the transplantation of adult BMMNCs compared with standard care alone. Another meta-analysis was reported by De Jong et al. [24], which included a total of 2,037 patients and 30 randomized controlled trials. The authors concluded that BMMNC treatment increased LV ejection fraction (EF), decreased LV end-systolic volume, and reduced the infarct size when compared with those in controls. A different message from this meta-analysis is that no functional improvements in cardiac function, volume, or infarct size were found, when cardiac magnetic resonance imaging (cMRI)-based measurement was used as an end-point for analysis.

**Mesenchymal stem cells (MSCs)**

Bone marrow was the first source reported to contain MSCs. Bone marrow contains a rare population of adherent cells. Initially, these cells were named stromal cells, but are now referred to as mesenchymal stem cells (MSCs) [25]. MSCs were first isolated from adipose tissue [26] and umbilical cord blood for clinical applications. Several reports published in the 1980s demonstrated that bone marrow-derived MSCs (BM-MSCs) could give rise to chondrocytes, osteoblasts, and skeletal muscle [27, 28]. In addition, cardiomyocytes can be generated from BM-MSCs [29]. The transplantation of MSCs from human bone marrow has been shown to improve LV function after myocardial infarction in rats [30]. On the basis of these findings, BM-MSCs have been used in clinical trials for both acute MI and chronic ischemic cardiomyopathy.

Recently, 2 large clinical trials have been reported. The POSEIDON study was undertaken to determine and compare the safety and efficacy between human allogeneic and autologous BM-MSCs in patients with ischemic cardiomyopathy [31]. Transplantation of BM-MSCs (20 million, 100 million, or 200 million cells) was performed by trans-endocardial injection into 10 LV sites. It was reported that allogenic BM-MSC-treated patients showed a low rate of immunologic reaction and reverse remodeling without significant improvement of LV function.

Another clinical trial, TAC-HFT, was performed; it was a randomized, double-blinded, placebo-controlled trial to compare the efficacy between BM-MSCs and BMMNCs [32]. This trial demonstrated a significant decrease in infarct size in the BM-MSC group, but not in the BMMNC or placebo group. Similarly, the regional peak circumferential strain on cMRI at the injection site improved in the BM-MSC group but not in the BMMNC or placebo group. However, LV chamber volume and ejection fraction did not differ between the BM-MSC and BMMNC groups.

**Peripheral blood-derived stem cells**

Circulating CD34 cell surface marker-positive endothelial progenitor cells (EPCs) have been isolated from peripheral blood [33, 34]. Experimental study in rats suggests that the transplantation of circulating blood progenitor cells (CPCs) affects neovascularization and the remodeling process of infarcted cardiac muscle [35]. It was shown that capillary density and neovascularization were significantly greater in rats transplanted with EPCs than in control rats. Enhanced neovascularization after the transplantation of EPCs led to a reduction in LV volume and preservation of cardiac contraction after MI. Following these investigations, a total of 59 patients in the TOPCARE-AMI trial with acute myocardial ischemia (AMI) were randomly assigned to receive either CPCs (n=30) or BMMNCs (n=29) after AMI [36]. The final results of this trial demonstrated that intracoronary infusion of CPCs or BMMNCs is feasible and safe in patients with AMI. In terms of efficacy, significant improvements in global LV function were also reported, as was a significant reduction in LV end-systolic volume, suggesting a favorable effect on the LV remodeling process over one year after cell infusion following AMI.

**Adipose-derived stem cells**

In 2001, adipose-derived stem cells were first identified as MSCs in adipose tissue [26]. Since then, adipose tissue has been studied as a cell source for regenerative therapy. Studies showing that adipose-derived MSCs can differentiate into cardiomyocytes and endothelial cells have prompted research in animal models of heart failure [37, 38]. In rats, transplanted adipose-derived stem cells were able to improve the cardiac function of infarcted hearts [39]; however, no clinical trials...
using adipose-derived stem cells have yet been reported \[40\].

**Umbilical cord blood stem cells**

Human umbilical cord blood (HUCB) has the ability to give rise to a population of cells in vitro exhibiting the characteristics of mesenchymal progenitors \[41\]. HUCB cells can be easily harvested, self-renew, proliferate, and differentiate into various lineages. Cardiac regeneration studies in rats with MI demonstrated that HUCB stem cells significantly improved cardiac function and performance \[42, 43\]. More recently, UCB stem cell transplantation into the right ventricle of juvenile pig hearts has been conducted and it was shown that the intramyocardial injection of UCB stem cells during an open chest procedure was technically safe \[44\]. On the basis of these lines of evidence, two clinical trials using UCB stem cells are currently ongoing to treat congenital heart diseases (Table 3).

**Resident cardiac stem cells**

Until recently, the fully differentiated heart has been believed to have no capability for cell turnover and self-repair of damaged myocardium. Cardiac stem cells (CSCs) within the heart tissue were first reported in 2002 \[45\]. It was demonstrated that the post-natal murine myocardium contains a resident side population of cells with stem cell-like activities. These cells were shown to constitute only 1% of total cardiac cells and had the potential to differentiate into cardiomyocytes. In 2003, two groups reported the successful isolation of CSCs from the rodent heart. These CSCs express three cell surface markers: stem cell antigen-1 (Sca-1), stem cell factor receptor (c-kit), and multidrug resistance protein-1 (MDR-1) \[46, 47\]. These CSCs were shown to be self-renewing, clonogenic, and multipotent, exhibiting the ability to differentiate into cardiomyocytes. In addition, it was reported that, upon intravenous or intramyocardial transplantation of CSCs after ischemia/reperfusion injury, these cells could target damaged myocardium and differentiate into cardiomyocytes with and without cell fusion in the host myocardium.

In 2004, a report was published describing the isolation of undifferentiated cells that grow as self-adhesive cultures, so-called “cardiospheres”, from postnatal atrial or ventricular biopsy specimens from human heart \[48\]. Three years later, a clinically applicable method for the isolation of human cardiac stem cells from endomyocardial biopsy was developed \[49\]. This involved modification of the culture method to obtain reasonable cell numbers of cardiosphere-derived cells (CDCs) for transplantation. Unlike CSCs that are purified based on the expression of c-kit, CDCs do not require cell sorting. Human CDCs are expressed in infant heart more than in adult heart; these cells are predominantly present in the right atrium and outflow tract of the heart and have a mesenchymal cell-like phenotype, such as CD105, CD90, CD29, CD73, CD71, and Stro-1, while they rarely express c-kit and do not express endothelial and hematopoietic cell surface markers \[50\]. The discovery of endogenous CSCs is one of the exciting new findings in the field of cardiac regeneration, which has attracted substantial interest relating to these cells’ proliferative and differentiation potential. During the last decade, several independent laboratories have revealed the potential of CSCs/CDCs for cardiac regeneration \[51-55\]. The results of these studies have revealed that CSC/CDC administration improves ventricular function and attenuates ventricular remodeling in animal models of acute and chronic ischemic heart failure.

**Table 2. Clinical trials of cell therapy using resident cardiac stem cells**

<table>
<thead>
<tr>
<th>Name of the trial</th>
<th>Study design</th>
<th>Number of patients</th>
<th>Delivery route</th>
<th>Cell type</th>
<th>Cell dose</th>
<th>Follow-up (months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCiPIO (NCT00474461)</td>
<td>open-label, randomized, controlled study</td>
<td>23 patients control (n=7); cell-treated (n=16)</td>
<td>IC</td>
<td>autologous c-kit CSCs</td>
<td>1×10⁶ cells</td>
<td>4 and 12 mo</td>
<td>↓LVEF [infarct size] NYHA class</td>
</tr>
<tr>
<td>CADUCEUS (NCT00893360)</td>
<td>open-label, randomized, controlled study</td>
<td>25 patients control (n=8); cell-treated (n=17)</td>
<td>IC</td>
<td>autologous CDCs</td>
<td>12.5-25 ×10⁶ cells</td>
<td>6 and 12 mo</td>
<td>↑viable mass [regional strain] infarct size</td>
</tr>
<tr>
<td>ALLSTAR (NCT01458405)</td>
<td>randomized, double-blind placebo-controlled study</td>
<td>estimated enrollment 274 patients</td>
<td>IC</td>
<td>allogeneic CDCs</td>
<td>25×10⁶ cells</td>
<td>ongoing</td>
<td></td>
</tr>
<tr>
<td>TICAP (NCT01273857)</td>
<td>open-label, prospective, nonrandomized study</td>
<td>14 patients control (n=7); cell-treated (n=7)</td>
<td>IC</td>
<td>autologous CDCs</td>
<td>3×10⁵ cells/kg</td>
<td>3 to 18 mo</td>
<td>↑RVEF ↓WAZ ↓NYUPHFI</td>
</tr>
<tr>
<td>PERSEUS (NCT01829750)</td>
<td>open-label, randomized, controlled study</td>
<td>34 patients control (n=17); cell-treated (n=17)</td>
<td>IC</td>
<td>autologous CDCs</td>
<td>3×10⁶ cells/kg</td>
<td>ongoing</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: IC: intracoronary; CSCs: cardiac stem cells; CDCs: cardiosphere-derived cells; LVEF: left ventricular ejection fraction; RVEF: right ventricular ejection fraction; WAZ: weight for age z-score; NYHA: New York Heart Association; NYUPHFI: New York University Pediatric Heart Failure Index.
administered 10^6 autologous c-kit CSCs by intracoronary injection. By contrast, 13 control patients received only CDC infusion via intracoronary administration in patients with myocardial infarction. The results of the CADUCEUS trial did not demonstrate significant changes in functional measurements of end-diastolic volume, end-systolic volume, and LVEF between groups. On the basis of these encouraging results, the ongoing ALLSTAR trial (NCT01458405) is being conducted to determine the safety and efficacy of allogeneic CDC infusion via intracoronary administration in patients with MI.

### Cardiac failure in congenital heart disease

The clinical outcomes of congenital heart disease including HLHS have dramatically changed due to major advances in the fields of pediatric cardiology, congenital heart surgery, and intensive care over recent decades. It was reported that 50-70% of infants born with HLHS are expected to reach adulthood [60]. Meanwhile, progressive late heart failure has become a serious problem and there is increasing recognition of the importance of heart failure in child health [3]. In the United States, there are currently 11,000-14,000 pediatric heart failure-related hospitalizations annually, with an overall mortality of 7% [61]. The majority of

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Table 3. Clinical trials and case reports of cell therapy for congenital heart diseases

<table>
<thead>
<tr>
<th>Target diseases</th>
<th>Number of patients</th>
<th>Age of patients</th>
<th>Follow-up (months)</th>
<th>Cell type</th>
<th>Delivery method</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLHS</td>
<td>1</td>
<td>11 mo</td>
<td>3</td>
<td>BMCs</td>
<td>IC</td>
<td>↑RVEF</td>
<td>Rupp et al. [51]</td>
</tr>
<tr>
<td>HLHS (TICAP trial: Okayama University Hospital)</td>
<td>14</td>
<td>5 mo - 3 y</td>
<td>18</td>
<td>CDCs</td>
<td>IC</td>
<td>↑RVEF, ↑WAZ, ↓NYHA, ↓NYPHF index</td>
<td>Ishigami et al. [52] (NCT01273857)</td>
</tr>
<tr>
<td>HLHS (Duke University)</td>
<td>20</td>
<td>0 - 2 day</td>
<td>12</td>
<td>UCB</td>
<td>IV</td>
<td>ongoing</td>
<td>(NCT01445041)</td>
</tr>
<tr>
<td>HLHS (Mayo Clinic)</td>
<td>10</td>
<td>0 - 18 mo</td>
<td>6</td>
<td>UCB</td>
<td>IM</td>
<td>ongoing</td>
<td>Burk et al. [53] (NCT01883076)</td>
</tr>
<tr>
<td>univentricular heart (PERSEUS trial: Okayama University Hospital)</td>
<td>34</td>
<td>6 mo - 6 y</td>
<td>12</td>
<td>CDCs</td>
<td>IC</td>
<td>ongoing</td>
<td>(NCT01829750)</td>
</tr>
<tr>
<td>dilated cardiomyopathy and congenital heart disease</td>
<td>9</td>
<td>4 mo-16 y</td>
<td>24 - 52</td>
<td>BMCs</td>
<td>IC</td>
<td>↑LVEF, ↑NYHA, BNP</td>
<td>Rupp et al. [54]</td>
</tr>
<tr>
<td>dilated cardiomyopathy</td>
<td>1</td>
<td>2 y</td>
<td>6</td>
<td>BMCs</td>
<td>IC</td>
<td>↑LVEF, ↑NYHA</td>
<td>Rup et al. [55]</td>
</tr>
<tr>
<td>dilated cardiomyopathy</td>
<td>2</td>
<td>6 y, 9 y</td>
<td>2 - 6</td>
<td>PSCs</td>
<td>IC</td>
<td>↑LVEF, ↑NYHA</td>
<td>Olguntürk et al. [56]</td>
</tr>
<tr>
<td>dilated cardiomyopathy</td>
<td>1</td>
<td>4 mo</td>
<td>4</td>
<td>BMCs</td>
<td>IM</td>
<td>↑LVEF</td>
<td>Lacis et al. [57]</td>
</tr>
<tr>
<td>dilated cardiomyopathy</td>
<td>2</td>
<td>3 mo, 4 mo</td>
<td>4</td>
<td>BMCs</td>
<td>IC</td>
<td>↑LVEF, ↑BNP</td>
<td>Rivas et al. [58]</td>
</tr>
<tr>
<td>dilated cardiomyopathy</td>
<td>6</td>
<td>4 mo -17 y</td>
<td>12</td>
<td>BMCs</td>
<td>IM</td>
<td>↑LVEF, ↓Ross</td>
<td>Bergmane et al. [59]</td>
</tr>
<tr>
<td>myocardial infarction</td>
<td>1</td>
<td>9 y</td>
<td>3</td>
<td>BMCs</td>
<td>IC</td>
<td>↑LVEF, NYHA</td>
<td>Limbsuwan et al. [60]</td>
</tr>
</tbody>
</table>

Abbreviations: HLHS: hypoplastic left heart syndrome; CDCs: cardiosphere-derived cells; UCB: umbilical cord blood; BMCs: bone marrow cells; PSCs: peripheral stem cells; IC: intracoronary; IM: intramuscular; IV: intravenous.

**Cardiac stem cell therapy for myocardial infarction**

Because of the developmental capability of cardiomyocyte differentiation, resident CSCs are thought to be the ideal stem cells, being superior to all other cell types. In addition, a recent report has demonstrated that CSCs had superior regenerative ability compared with BMMNCs, BM-MSCs, and adipose-derived stem cells [56]. The study design of recent resident CSC-based cardiac regenerative therapy and the following results are summarized in Table 2. To date, three phase 1 clinical trials using resident CSCs have been completed and reported. The SCPIO study (NCT00474461), reported by Bolli and Anversa et al., was the first-in-human phase 1 randomized controlled trial of intracoronary autologous c-kit CSC infusion for ischemic heart failure. A total of 33 patients (20 CSC-treated and 13 control subjects) were enrolled in this study. Autologous c-kit CSCs were isolated from the right atrial appendage during cardiac surgery and purified based on the expression of c-kit. About 4 months after bypass surgery, CSC-treated patients were administered 10^6 autologous c-kit CSCs by intracoronary injection. By contrast, 13 control patients received only standard care alone. Although the 2-year follow-up results have not been published, the interim results of the SCPIO trial are quite impressive [57]. The patients treated with autologous c-kit CSCs showed an increase in LVEF measured by echocardiographic analysis (30.3% to 38.5%) at 4 months after cell infusion; meanwhile, LVEF did not change in the control patients. This functional improvement of LV was sustained at 1 year of follow-up. cMRI analyses of ventricular function in this trial were described by Chugh et al. [58]. They showed that CSC injection led to improvement in both global and regional LV function, a reduction of scar size, and an increase in viable cardiac tissue.

Another randomized study, the CADUCEUS trial (NCT00893360), has been reported by Makkar and Marban et al. [59]. This study consisted of 25 patients at 2 to 4 weeks after MI (17 CDC-treated and 8 control subjects). Autologous CDCs from endomyocardial biopsy specimens were administered through intracoronary injection 1.5 to 3 months after MI. This procedure was safe and caused a reduction of scar size, and improvement of regional cardiac contraction. The authors of the CADUCEUS trial did not demonstrate significant changes in functional measurements of end-diastolic volume, end-systolic volume, and LVEF between groups. The results of the CADUCEUS trial did not demonstrate significant changes in functional measurements of end-diastolic volume, end-systolic volume, and LVEF between groups. On the basis of these encouraging results, the ongoing ALLSTAR trial (NCT01458405) is being conducted to determine the safety and efficacy of allogeneic CDC infusion via intracoronary administration in patients with MI.

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these children have congenital heart diseases, although these include a significant number with cardiomyopathy or myocarditis. When heart failure is established in children for more than 1 month in duration, poor weight gain becomes evident, and in the longer term, failure in linear growth can be manifested. This “failure to thrive” is a clinical feature of heart failure [62]. Even with the best supportive medical therapy, many children with heart failure will progress to the irreversible stage. Heart transplantation is an option for children with end-stage heart failure. However, the supply of available organs remains small relative to the number of patients. In the United States, fewer than 400 pediatric heart transplantations are performed annually, whereas the mortality rate for those waiting for heart transplantation remains high [63]. To improve the clinical outcomes of end-stage heart failure in children, advanced technologies are strongly desired.

Evidence for cardiac regeneration in children

Cardiac regeneration in lower vertebrates, for example, amphibians and zebrafish, has been demonstrated [5]. Even in mammals, surgical resection of the ventricular apex in 1-day-old mice stimulates a regenerative response that appears to restore the damaged heart to its normal anatomy and function [64]. The number and functionality of resident CSCs are significantly different in children compared with those in adults. The resident CSCs are most abundant in the neonatal period, but decline rapidly with age [65, 66]. In addition, these resident CSCs can be isolated from a variety of congenital heart diseases, including those in the neonatal and adolescent periods [66]. When human CSCs were transplanted into infarcted myocardium of immunodeficient rats, neonatal-derived CSCs had a significantly higher ability to improve cardiac function and to prevent adverse remodeling compared with adult-derived CSCs [4]. These novel findings of human CSCs have the potential to contribute to a new therapy for congenital heart failure patients.

Cardiac stem cell therapy for congenital heart disease

Clinical evidence of cardiac regeneration in children can be seen in patients with an anomalous origin of the left coronary artery from the pulmonary artery (ALCAPA). Most children with ALCAPA present with severely impaired cardiac function. Some children have very high serum troponin levels, which indicates the loss of cardiomyocytes [67]. After corrective surgery (aortic implantation of the left coronary artery), the clinical outcome is very good and left ventricular function improves dramatically [68, 69].

As mentioned above, regenerative capacity seems to be superior in children compared with that in adults. However, almost all cardiac regeneration studies have been reported in adults. To date, there have been no large clinical trials in children using any type of cell-based therapy and only case report series are available [70-78]. As shown in Table 3, most of the children receiving cell therapy had dilated cardiomyopathy (DCM) with a critical clinical status.

In 2009, Rupp et al. reported the first case of cell-based therapy in a child with DCM [73]. They reported that the intracoronary administration of BMMNCs was technically feasible. After cell transplantation, the clinical condition of the child improved and the child could be discharged without any adverse events reported. The LVEF increased from 24%
to 41% three months later and serum BNP levels decreased in association with an improvement of NYHA functional classification. The same group reported a case of cell therapy for an 11-month-old boy with HLHS in 2010 [70]. This boy presented with sustained heart failure and stem cell infusion before heart transplantation was planned. Intracoronary infusion of autologous BMMNCs was performed without any complications or ischemic reaction. Three months after cell therapy, cardiac function improved with marked reduction of end-diastolic and end-systolic volumes. Another two cases of autologous BMMNC infusion for children with congenital heart failure were also reported [72]. Pooled data analysis of surviving patients from these case reports might be difficult because of the different diseases, including DCM and congenital heart malformations. However, all surviving children without heart transplantation had an overall increase in EF of 25.7% to 43.1% and improvement in clinical conditions [77].

In 2014, the TICAP trial (NCT01273857) was reported by Ishigami and Oh et al. [79]. Unlike the SCIPIO and the CADUCEUS trials, the TICAP study was the first designed to treat children with single right ventricle circulation caused by HLHS. A total of 14 patients (7 CDC-treated and 7 control subjects) were enrolled. Autologous CDCs were isolated during a shunt procedure, such as stage 2 bidirectional cavopulmonary connection or stage 3 total cavopulmonary connection (Figure 1A). Intracoronary infusion of autologous CDCs was performed 4 to 5 weeks after the cardiac surgery (Figures 1C and D). In this trial, we demonstrated not only the safety and feasibility of CDC infusion for children with HLHS but also the efficacy of CDC injection. At 18 months of follow-up, right ventricular function in the CDC-treated patients showed a significant improvement from an average baseline value of 46.9% to 54.0%, whereas no change was found in the controls. Heart failure status assessed by New York University Pediatric Heart Failure Index (NYUPHFI) [80] improved in the CDC-treated patients. Growth failure is a clinical feature of heart failure [62, 81], and interestingly, we found significant somatic growth (WAZ; weight for age z-score) in the CDC-treated group. Growth failure is a clinical feature of heart failure [62, 81], and interestingly, we found significant somatic growth (WAZ; weight for age z-score) in the CDC-treated group. On the basis of the results of this phase 1 trial, we are now conducting a randomized-controlled, prospective phase 2 trial. Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease (PERSEUS: NCT01829750) is designed to assess the efficacy of CDC infusion in children with single ventricle physiology. In this trial, a total of 34 patients are randomly assigned to the treatment or a control group at a 1:1 ratio [82].

Our TICAP phase 1 trial is the first clinical study in patients with congenital heart disease [79]. There are another two clinical trials using autologous umbilical cord blood (UCB) stem cells, as shown in Table 3. The first case in the Mayo Clinic of direct intramyocardial injection of UCB-derived mononuclear cells in an infant with HLHS was reported recently [71]. UCB was collected at the time of delivery and the mononuclear cell fraction was stored. At the stage 2 palliation, the cells were transplanted into the right ventricle free wall. No bleeding, arrhythmia, or hemodynamic changes occurred during the cell injections. Echocardiography analysis at 3 months after transplantation showed an improvement in RVEF of 35% to 50%. Future studies and longer follow-up are required to evaluate the efficacy and safety of intramyocardial injection of UCB-derived stem cells in HLHS.

Unresolved issues

Cell retention and engraftment

One of the major problems associated with cell therapy is the low rate of donor cell retention and engraftment in the recipient heart, which is caused by the early “wash-out” of cells from coronary blood flow and heart contraction. The rate of acute cell retention (<24 h) in the heart is usually <10%, regardless of the cell type and delivery route [83-85]. Low survival and poor engraftment of stem cells greatly limit their therapeutic efficacy. To resolve these problems, several innovative new technologies including biomaterial delivery vehicle and magnetic targeting technology have been applied to cell-based therapy.

Several studies related to injectable hydrogel therapy and delivery strategies to improve cell retention have been discussed [86-88]. Transplanted human CDCs with the addition of basic fibroblast growth factor (bFGF) were administered in a slow-release hydrogel to immunosuppressed pigs [89]. In this study, it was demonstrated that controlled delivery of bFGF had an additive effect when combined with human CDCs, resulting in enhanced cell engraftment and cardiac function. In 2014, a first-in-man study with the intracoronary delivery of acellular injectable bioabsorbable scaffold (IK-5001) after ST segment elevation MI was conducted [90]. It was shown that intracoronary deployment of bioabsorbable scaffold is feasible and tolerated. The same investigators launched a multicenter, randomized, controlled double-blind trial to assess the safety and efficacy of IK-5001 for the prevention of ventricular remodeling (NCT01226563). Biomaterial-based cell delivery approaches may be promising for the future development of efficient delivery techniques.

Another technology aimed at cell retention is magnetic targeting. Stem cell labeling with iron oxide particles allows not only the detection of labeled cells by cMRI but also the
enhancement of cell retention by magnetic induction[91-93]. In animal models, CDCs can be loaded with superparamagnetic iron oxide nanoparticles (SPIONs) without interfering with their viability. Compared with non-targeted CDCs, magnetically guided CDC delivery into the heart can boost short-term cell retention, long-term engraftment, and the functional benefit of the cell therapy [92]. Following the success of magnetic cell delivery in various animal models, this simple but novel technique to improve injected cell retention is readily generalizable and offers the potential for rapid translation to clinical applications using the FDA-approved SPIONs.

**Mechanism of stem cell therapy for heart failure**

Although accumulated evidence has suggested the efficacy of cell-based cardiac regenerative therapy, the mechanisms of the beneficial effects remain largely unresolved and under intense investigation. Initially, stem cells were believed to promote cardiac regeneration via direct differentiation into cardiomyocytes [94]. More recent studies have revealed that cell transplantation stimulates an endogenous cardiac repair process via paracrine signaling, direct cell-to-cell interaction, and/or transfer of microRNAs that influence the transcriptional activity of host cells [95-97]. The relative role of direct differentiation versus the paracrine effect of human CDCs in MI mice showed that human CDCs release growth factors in the tissue, reduce apoptosis, and increase both viability and perfusion in heart tissue. Direct differentiation occurs; however, it accounts for only 20% to 50% of the overall increases in capillary and cardiomyocyte density. A paracrine effect contributing to the beneficial results of stem cell-based therapy promotes the hypothesis that the combination of chemokines with cell therapy may be synergistically effective for cardiac regeneration [98].

Therapeutic interventions using growth factor administration are the focus of future investigation [99]. Various experimental and clinical trials have suggested that growth hormone (GH) and insulin-like growth factor 1 (IGF-1) play important roles in cardiac growth and function. In addition to GH itself, GH-releasing agonist and GH-releasing peptides such as ghrelin are also thought to have a cardioprotective role [100]. These hormonal mediators also play an important role in stem cell function. Resident cardiac stem cells express GH-releasing hormone receptor (GHRH-R) and synthetic agonists of this receptor promote the proliferation and survival of resident cardiac stem cells [101].

**Administration route**

Although the major routes for cell transplantation are direct injections into the ventricle wall (trans-endocardially or trans-epicardially) and intracoronary infusion, the most effective methods for cell delivery remain unknown. Several studies have investigated the therapeutic potential among various delivery routes. A study with radiolabeled cells demonstrated that intramyocardial injection is more efficient than intracoronary delivery in swine models of infarction [102]. However, this method is limited by the requirement for surgery as well as the fact that repeated injection is difficult due to invasiveness.

A percutaneous trans-arterial approach has been used for both intramyocardial injections and intracoronary injection [103]. Three-dimensional NOGA endocardial mapping with electromagnetic-guided percutaneous intramyocardial therapy is a novel approach that is currently being used in clinical trials for the real-time assessment of myocardial viability and for the delineation of the infarct and infarct border zone [104].

Intracoronary infusion is the most popular mode of cell delivery in many clinical trials. This technique is similar to that used for coronary intervention, which involves positioning of an angioplasty balloon in one of the coronary arteries. The stem cells are infused during coronary blood flow stopped by balloon inflation [105]. The safety of this method has been confirmed in multiple clinical trials [57, 59, 79]. Intravenous delivery is a minimally invasive and attractive approach for cardiac regeneration; however, poor delivery efficiency by the entrapment of donor cells mostly in the lungs is a problem that needs to be overcome [106]. Despite the disadvantage of low cell retention, intracoronary and intravenous injections are capable of being performed repeatedly. Combined strategies may resolve these problems related to the low retention of stem cells by intracoronary and intravenous delivery.

**Cell type (autologous or allogeneic?)**

As we described previously, multiple sources of stem cells have been applied for cardiac regeneration. The vast majority of clinical trials were conducted with autologous cells. This approach avoids immunologic rejection; however, it necessitates patient-specific tissue harvesting, cell processing, and quality control, resulting in a few weeks’ delay of treatment and possible variations in cell potency related to patient age and disease [107]. The use of allogeneic cells, if safe and effective, may resolve these limitations; however, immune rejection could be the next stage required for investigation. Allogeneic CDC therapy without immunosuppression in rats has been reported to be safe and induced only a mild transient local response without signs of systemic immunogenicity. Despite lower long-term
engraftment compared with syngeneic cells, allogeneic CDC transplantation produced similar beneficial structural and functional effects after infarction [108]. This work motivates the testing of allogeneic human CDCs as a potential clinical product for cardiac regeneration. The ongoing ALLSTAR trial (NCT01458405) is intended to determine the safety and efficacy of allogeneic CDCs administered by intracoronary infusion in patients after myocardial infarction.

Future perspectives

To enhance cell retention and engraftment using less invasive techniques, we have now reached the next generation of stem cell-based strategies. The key to optimal cardiac regenerative therapy is to combine strategies including priming stem cells, addition of growth factors, and combination with biomaterials. In pediatric cardiac regeneration, a less invasive catheter technique for tissue harvesting could be applicable (Figure 1B). Transcoronary CDC infusion can be performed in a repeated fashion, for example, inter-stage of palliation and even after stage 3 operation, to enhance cardiac function.

Conclusions

Several types of stem cell have been identified and investigated in clinical trials with positive results. In the field of congenital heart diseases, the results of the TICAP trial are promising. However, stem cell therapy for children with heart failure has just begun and further studies will be required to understand the basic cell biology in order to enhance their regenerative actions by using combined strategies with new tissue engineering technologies. Although there are many hurdles to overcome, we believe that the future prospects for cardiac regeneration in children are bright. The positive results from the TICAP trial could be a breakthrough for cardiac regeneration therapy in children; however, we urgently need sufficiently powered, randomized trials to answer the questions regarding CDC transplantation in patients with various types of congenital heart disease.

Conflict of interest

The authors declare that there are no conflicts of interest.

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