Regenerating the ailing heart: Stem cell therapies for hypoplastic left heart syndrome

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ABSTRACT

Hypoplastic left heart syndrome (HLHS) is a complex congenital heart defect (CHD) characterized by a spectrum of underdeveloped left-sided cardiac structures. It is a serious defect and warrants either 3-staged surgical palliation or a heart transplant. Despite numerous surgical advancements, long-term outcomes remain challenging and still have significant morbidity and mortality. There have been notable advancements in stem cell therapy for HLHS, including developments in diverse stem cell origins and methods of administration. Clinical trials have shown safety and potential benefits, including improved ventricular function, reduced heart failure, and fewer adverse events. Younger myocardium seems particularly receptive to stem cell signals, suggesting the importance of early intervention. This review explores the potential of emerging stem cell-based therapies as an adjunctive approach to improve the outcomes for HLHS patients.

Keywords: Hypoplastic left heart syndrome, stem-cell therapy, uni-ventricular pathology

INTRODUCTION

Hypoplastic left heart syndrome (HLHS) is a group of heterogeneous congenital cardiac malformations characterized by hypoplasia of the left ventricle and systemic outflow obstruction, which leads to varying degrees of aortic and cardiac malformations and impairment of left ventricular (LV) contractility.[1] The key anatomic features characteristic of HLHS are abnormally widened pulmonary trunk and orifice and narrowed ascending and transverse aorta and aortic orifice without transposition of the vessels. It is the most prevalent single-ventricle heart defect in the US, affecting 1 in 3800 newborns,[2] and constitutes 2%–3% of congenital heart defects (CHDs), leading to 25% of deaths related to heart ailments within the 1st week of the neonatal period. HLHS shows an increased prevalence in males, with a male-to-female ratio of 2.70,[3] and is invariably fatal in 100% of cases without prompt treatment. The etiology of HLHS is multifactorial and is associated with chromosomal disorders such as Turner syndrome, DiGeorge syndrome, and Down syndrome and genetic causes such as mutations in genes encoding signaling pathways and transcription factors that hinder the proliferation, differentiation, and maturation of cardiac progenitor cells (CPCs), resulting in underdevelopment of the left heart structures and diminished contractility of cardiomyocytes.[4] Maternal obesity and maternal diabetes are some of the nongenetic factors that influence the development of HLHS.[5] Researchers have also hypothesized that altered blood flow is responsible for the underdevelopment of cardiac

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structures seen in HLHS.[6] These factors, along with mutations, are shown in Figure 1. Infants diagnosed with HLHS require both a patent ductus arteriosus and an interatrial connection, such as an atrial septal defect, to ensure survival until surgical intervention is possible. Consequently, immediately after birth, a continuous infusion of prostaglandin E1 is administered to uphold the patency of the ductus arteriosus.[7] Following this, surgical intervention is required, which involves a three-stage palliative surgical approach, in which initially, the Norwood operation is performed in the 1st week of life. Subsequently, a bidirectional cavopulmonary anastomosis or the Glenn procedure is performed at 4–6 months of age, with the final stage being the Fontan procedure at 16–18 months that enables the right ventricle (RV) as the primary pump to compensate for the underdeveloped LV.[8] The advancements in multi-stage surgical management have enhanced survivability in HLHS patients from 0% to 56% in term infants,[9] but these result in complications such as cardiac scarring, arrhythmias, thromboembolisms, and a progressive decline in functional status. This deterioration can lead to end-organ damage, including cirrhosis and protein-losing enteropathy, and unfortunately, approximately one-third of HLHS patients ultimately risk succumbing to RV failure by the age of 25.[10] At present, cardiac transplantation, with a 5-year survival rate of approximately 70%, is the sole recourse for individuals in end-stage cardiac failure and certain cases of HLHS, but it carries the inherent risks of prolonged immunosuppression and graft dysfunction with its feasibility significantly restricted by the limited availability of donors.[11] Due to the limitations of standard management of HLHS, stem cell therapy, as an experimental procedure, offers hope for individuals with HLHS with the concept of delivering isolated cells to the site of injury or lesion for repair and regeneration. Several types of stem cells are currently being explored for their potential application in treating HLHS.[8] Recent clinical trials have demonstrated the promising potential of stem cell therapy in mitigating long-term complications arising from surgery, improving the overall quality of life, and reducing mortality rates associated with various heart diseases. This article explores the emergence of stem cell treatments and their potential role in conjunction with traditional surgical approaches for HLHS, thus highlighting the next-generation modalities for the treatment of HLHS.

STEM CELLS AND THEIR CHARACTERISTICS

Stem cells are specialized cells that can form different types of functional tissue and can be subdivided into pluripotent cells or embryonic stem cells (ESCs), and adult stem cells or somatic cells based on their origin.[12] Pluripotent stem cells are derived from the inner mass of the blastocyst, an early stage of the embryo, and can differentiate into any cell type(s), while somatic stem cells are tissue specific and can generate most cell types of a specific organ. The term induced-pluripotent stem cells (iPS cells) means reprogrammed adult stem cells with similar characteristics to an ESC and capable of producing all bodily cell types.[13] Mesenchymal stem cells (MSCs) are stromal cells derived from umbilical cord, adipose tissue, bone marrow, endometrial polyps, or menstrual blood [Figure 2] and can be phagocytic,

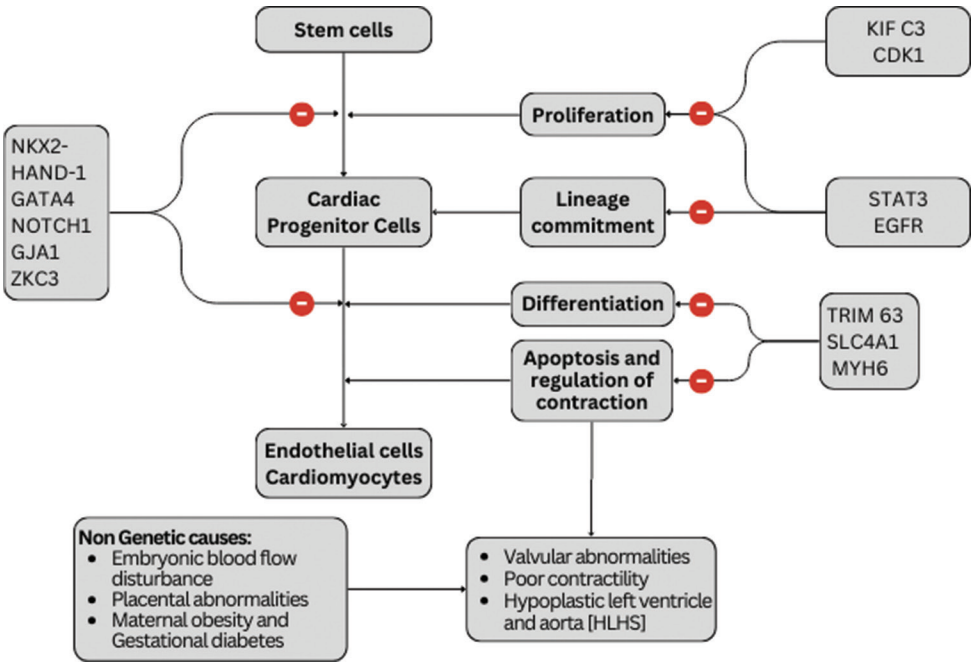

Figure 1: Various hypotheses for hypoplastic left heart syndrome

immune suppressors, support T-cell cytotoxicity and/or express major histocompatibility complex II, and are thus, ideal for therapeutic management of CHDs.[14,15] The characteristics of various types of stem cells being researched in human studies of HLHS, such as MSCs, umbilical cord blood (UCB) cells, C-kit+ CPCs, and cardiosphere-derived cells (CDCs), are described in Table 1. The UCB cells are derived at birth and give rise to both hematological and nonhematological precursors and can differentiate into classical mesenchymal lineages and various other cell types.[16] CDCs originate from myocardial tissue cultured on poly-d-lysine and are specialized cells consisting of an inner core of undifferentiated cells surrounded by an outer shell of cells that are committed to differentiation into cardiomyocytes. They are defined by a phenotypic profile of CD105 positivity of >90%, and when transplanted, differentiate *in vivo* into cardiomyocytes, smooth muscle cells, and endothelial cells.[17] CPCs typically express C-kit, a receptor tyrosine kinase, and do not express CD45, Lin, a common marker of hematopoietic cells, or tryptase (a marker of mast cells) on their surface.[18]

ROLE OF STEM CELLS AND EXAMPLES FROM ANIMAL STUDIES

The principle of stem cell therapy is rooted in the belief that their delivery at the site of damage or injury can cause regeneration or healing,[19] possibly by the release of paracrine factors, mitochondrial transfer, exosomes/extracellular vesicles, and reconstitution of cardiac niche and also play an antifibrotic and anti-apoptotic role.[20] Stem cells can differentiate into cardiomyocytes, prevent inflammation, and stimulate angiogenesis, thus helping to reverse or attenuate deleterious remodeling, and with recent advancements such as exosomes and mitochondrial transfers, may further promote intercellular communication and rejuvenate endogenous cells. They can be administered by intracoronary or intramyocardial routes, and their effects can be further potentiated by preconditioning and genetic modification, resulting in an increased number of functional cardiomyocytes and improvement in RV function.[21] The possible mechanisms of stem cell therapy are depicted in Figure 3.

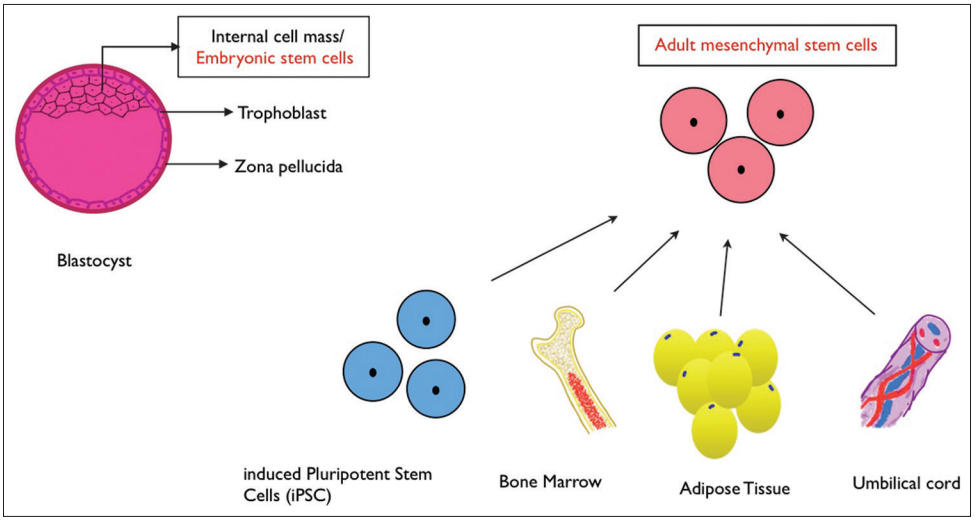

Figure 2: Types of stem cells and their origin

Table 1: Different types of stem cells being researched for hypoplastic left heart syndrome

Type of stem cells	Human mesenchymal cells	UCB cells	CDC	C kit + CPC
Origin	Bone marrow stromal cells	UCB at birth	Myocardial tissue cultured on poly-d-lysine	Epiblast of developing heart tubes
Give rise to	Mesodermal tissues, including bone, cartilage, muscle, tendon, ligament, and adipose tissue	Hematopoietic and nonhematopoietic cells	Cardiomyocytes, smooth muscle cells, and endothelial cells	Cardiomyocytes
Characteristic features	Clonogenic expression of CD105, CD73, CD90, CD29, and CD166 reduced expression of MHC-1 and absence of MHC-2, resulting in immunomodulatory role	Isolation of MSCs from UCB can differentiate into classical MSC lineages (bone, cartilage, and fat), hepatocyte-like cells, neuroglial-like cells, respiratory epithelial cells, and cardiomyocytes	Phenotypic profile of CD105 positivity of >90%	C kit positive Lack of CD45 tryptase

UCB: Umbilical cord blood, CDC: Cardiosphere-derived cell, CPC: Cardiac progenitor cell, MHC: Major histocompatibility complex, MSC: Mesenchymal stem cell

In HLHS, the effects of subclinical RV dysfunction are more pronounced due to under-development of the left side of the heart, leading to gross structural and microscopic changes in RV resulting in pressure and volume overload, and are often exacerbated by surgeries and ongoing physiologic demands.[22] Preclinical studies have been performed on animal models that produced RV dysfunction by various mechanisms to investigate the role of stem cells in heart diseases. In one such study on the porcine model of RV dysfunction by pressure overload produced by banding the main pulmonary artery, human MSCs and C-kit + CPCs were injected, resulting in decreased RV dilation and preserved RV function, along with reduced myocardial fibrosis and increased angiogenesis, cardiomyocyte and endothelial cell proliferation.[23] This is attributed to the effects of growth differentiation factor-15, belonging to the family of transforming growth factor-β, which reduces hypertrophic changes due to pressure overload. In another neonatal ovine pulmonary artery model, human UCB cells were used, which resulted in improved end-systolic elastance and preload-recruitable stroke work.[24] In a volume overload-induced RV dysfunction model, transplanted autologous UCB-mononuclear cell (MNC) improved RV diastolic function and increased angiogenesis.[25] These studies have highlighted the potential roles of stem cells in various models of RV dysfunction, which are relevant to single-ventricular pathophysiology as observed in CHDs like HLHS. However, the lack of engraftment of transplanted cells and the disproportionately low functional improvement are practical challenges in stem cell therapy-based treatment, which may limit their efficacy and usage; nonetheless, these challenges can be overcome by secretomes and proteomics as observed in some animal models, which was found to be as effective as live cell transplant.[26] Multiple studies support that the secretomes of stem cells are the primary cause for clinical benefits seen in many CHD and Myocardial infarction (MI) animal models. In a study by Sharma *et al.*, a dose of total condition medium from CPCs of neonates was better at improving the cardiac function in rat MI models when compared to live transplanted neural Crest-derived progenitor cells (nCPCs).[27] The evolution of more novel stem cell therapeutics may be based on the benefits of molecular mechanisms and their secretomes rather than mere stem cell engraftment.

PRECLINICAL AND CLINICAL TRIALS

A three-staged surgical intervention, which includes the Norwood procedure, bidirectional cavo-pulmonary shunt (Glenn procedure), and the Fontan procedure, has been the most recommended approach for years in terms of managing HLHS, but these surgical procedures can cause scarring leading to myocardial fibrosis and weakness.[7] Although these procedures increase survival rates, a cohort study of 27 HLHS patients done by Williams *et al.* reported an increased strain on the RV >8.7% (P = 0.0004) 1 month after the Norwood procedure, leading to death or RV dysfunction, ultimately requiring a heart transplant.[8] Therefore, superior protocols and advanced management techniques are becoming a necessity in the treatment of HLHS, and with multiple stem cell studies in adults and animals showing promising results, the utilization of these modalities is required to be investigated further. A study

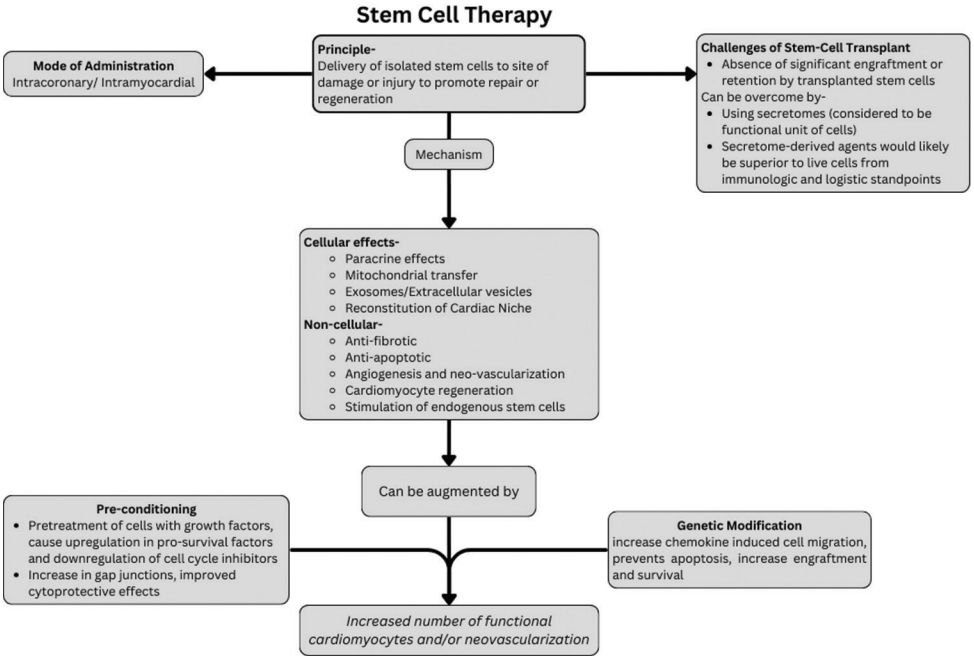

Figure 3: Mechanism of stem cell therapy

by Kobayashi *et al.* analyzed HLHS-derived iPS cells along with control iPS cells and human ESCs and reported that the HLHS-derived iPS cells had diminished differentiating capabilities into cardiomyocytes, defective sarcomeric organization, impaired calcium transient patterning, and altered responses to β-adrenergic antagonists.[28] An adult myocardial infarction study found that injection of stem cells to an area of injury led to <5% retention in the injury site. However, the patients benefited as they promoted neovascularization, paracrine signaling factors, and functional cardiomyocytes.[7]

Currently, there are multiple ongoing clinical trials assessing the best methods and sites of administration of stem cells, determining their safety, feasibility, and efficacy, and comparing stem cell therapy alone or in conjunction with surgical management. Several studies have suggested that children are more receptive to stem cell signals due to the plasticity of the myocardium in early developmental stages.[29] This seems to be supported by the fact that the turnover rate of myocardium falls from 1% in young adults to <0.45% by the age of 75 years,[30] and similarly, the cell cycle activity is lost rapidly, declining to negligible levels by the age of 20 years.[31] A phase 1 clinical trial exhibited safety in neonates who received autologous cord blood mononucleated cells during the Norwood procedure via intra-coronary infusion at cardioplegic arrest on days 2–3 of life.[32] A meta-analysis including animal and human studies reported that cell-based therapies improved ejection fraction (EF) in animals (MD = 6.9%; 95% confidence interval [CI] [4.24–9.55]; $P < 0.01$) and preserved EF in humans (MD = 4.84; 95% CI [1.62–8.07]; $P < 0.05$) when compared to controls, and also reported fewer adverse events in patients with cell-based therapy in comparison to controls (44/492 vs. 98/606, Peto odds ratio = 0.17; 95% CI [0.09–0.30]; $P < 0.01$).[33] A phase 1 clinical trial with 10 HLHS infants reported no death or significant safety concerns during a follow-up for over 6 months when stem cells derived from cord blood were injected during the Glenn surgery.[34] A randomized clinical study with 20 patients enrolled in either MSC injections or standalone surgical care hypothesized that MSCs improve and preserve RV function in young myocardium; however, the results of this study are still pending.[35] A study focusing on trans coronary infusion of CPCs using the stop-flow technique in children reported transitory events such as ST-segment elevation/depression, coronary artery vasospasm, bradycardia, and hypotension during the procedure, but was overall safe and feasible.[36] A randomized double-blinded juvenile porcine study evaluated intramyocardial injection of autologous UCB-MNCs versus control (dimethyl sulfoxide 10%) in RV and monitored their long-term safety and feasibility. At the end of the 3-month study, they reported that autologous UCB-MNCs can be collected safely and administered surgically in a pediatric setting for patients with CHD.[37] Patients who underwent 2- or 3-staged surgical palliations alone or received intracoronary CDCs were monitored for 36 months, following which they reported that those who received CDC infusions had improved RV EF (+8.0% ±4.7% vs. +2.2% ±4.3%; $P = 0.03$), reduced brain natriuretic peptide levels ($P = 0.04$), and reduced unplanned catheter interventions ($P = 0.04$) when compared to controls.[38] A phase 2 clinical trial by Ishigami *et al.*, with primary and secondary outcome measures being the assessment of cardiac function at 3 months, somatic growth, ventricular function, heart failure status, and quality of life at 12 months, reported a significant improvement in ventricular function in the CDCs group versus the control group (+6.4% [standard deviation (SD) 5.5] vs. +1.3% [SD 3.7]; $P = 0.003$); 17 of the total controls received a late CDC infusion and had increased ventricular function when compared to their baseline (38.8% [SD 3.7] vs. 34.8% [SD 7.4]; $P < 0.0001$). At the end of 1 year, the study reported overall ventricular function improvement (41.4% [SD 6.6] vs. 35.0% [SD 8.2]; $P < 0.0001$), decreased heart failure status ($P < 0.0001$) and cardiac fibrosis ($P = 0.014$), greater somatic growth ($P < 0.0001$) and higher insulin-like growth factors and hepatocyte growth factor when compared to their baseline.[39] The above studies are summarized in Table 2.[32,34-36,38-42]

LIMITATIONS OF STUDIES

The translation of findings from stem cell therapy studies for HLHS into clinical application faces several challenges. First, early-phase studies, such as the ones by Brizard *et al.*[32] and Burkhart *et al.*,[34] have had low patient recruitment numbers and limited sample sizes, thus having less generalizability. In addition, studies like Tarui *et al.*[38] have been limited by their small, nonrandomized designs, lack of blinding among cardiac interventionists, and a potential imbalance in preregistration variations between treatment groups. These studies also had a mixture of patients with different surgical histories, such as those after the Fontan operation and the bidirectional Glenn procedure, further complicating the interpretation of results. Furthermore, limitations highlighted in studies by Ishigami *et al.*[39,41] point toward the challenges in evaluating health-related quality of life, exercise capacity, neurodevelopmental outcomes, and long-term observations, particularly in the absence of appropriate control groups or comprehensive outcome assessments. Even ongoing trials, such as the one by Kaushal *et al.*,[42] face hurdles such as limited follow-up periods and the absence of injections into the RV in control groups, which can affect the validity of comparisons. Moreover, the collective absence of long-term studies and short follow-up durations across the studies emphasizes the need for longer observation over decades to fully comprehend the safety, efficacy, and

Table 2: Summary of clinical trials

Clinical trial with phase/ study	Objective	Number of subjects	Type of stem cells used	Mode of administration intracoronary/ intramyocardial	Results and conclusion
Phase 1 Brizard *et al*., 2023[32]	Safety and feasibility of adjunct autologous cord blood stem cell therapy during the Norwood heart operation	16	Autologous CB stem cell	IC	Safe and feasible infusion of autologous cord blood stem cells via novel intracoronary technique at cardioplegic arrest on days 2–3 of life during Norwood palliative surgery
Phase 1 Burkhart *et al*., 2019[34]	Safety and feasibility of intraoperative IM injections	10	UCB-MNCs	IM	Delivering during planned stage 2 surgical palliation for HLHS was safe and feasible preservation of baseline right ventricular function throughout follow-up and normalized growth rates support the design of a phase 2b follow-up trial
Kaushal *et al*., 2017[35]	Phase 1 - whether MSC injection will be both safe and feasible or to report any new major adverse cardiac events Phase 2 - efficacy hypothesis that MSC injection improves cardiac function compared to surgery alone	Phase 1-10 Phase 2-20	LMSCs	IM	Ongoing trial; expected to be complete by the end of 2025
Phase 1 and Phase 2 Eitoku *et al*., 2018[36]	Transcoronary transfer of cells with stop flow technique	Phase 1-7 patients with HLHS Phase 2-41 patients with single ventricular pathology	Autologous CDC	IC	Successfully completed, the therapy was declared to be safe and feasible
Tarui *et al*., 2015[38]	To assess midterm safety and clinical outcomes of intracoronary infusion of CDCs	14	CDCs	IC	Greater improvement in RVEF in infants receiving CDCs than in controls at 36 months (+8.0%±4.7% vs. +2.2%±4.3%; *P*=0.03). These cardiac function improvements resulted in reduced BNP levels (*P*=0.04), lower incidence of unplanned catheter interventions (*P*=0.04), and higher weight-for-age *Z* score (*P*=0.02) at 36 months relative to controls
Phase 2 Ishigami *et al*., 2017[39]	Whether it improves cardiac function in single ventricular pathology	41	Autologous CDC	IC	Reverse remodeling may improve heart failure status, somatic growth, and quality of life in patients and reduce parenting stress for their families
Burkhart *et al*., 2015[40]	Case report During stage 2 surgical palliation	1 infant	UCB-MNCs	IM	Progressive improvement in the RVEF during the 3-month interval
Kaushal *et al*., 2022[42]	Phase 1 - assess the feasibility, safety, and potential efficacy of CPC therapy Phase 2 - early assessment of the efficacy of nCPCs versus the current standard of care	Phase 1-10 Phase 2-11	Autologous nCPCs	IM	Ongoing trial; expected to be complete by mid-2025
Ishigami *et al*., 2015[41]	Whether intracoronary delivery of CDCs is feasible and safe	14 patients	Autologous CDCs	IC	Higher RVEF (31.5±6.8% vs. 40.4±7.6%; *P*=0.049), improved somatic growth (*P*=0.0005), reduced heart failure status (*P*=0.003), and lower incidence of coil occlusion for collaterals (*P*=0.007)

MSC: Mesenchymal stem cell, IC: Intracoronary, IM: Intramyocardial, CDCs: Cardiosphere-derived cells, CB: Cord blood, LMSCs: Longeveron MSCs, RVEF: Right ventricular ejection fraction, HLHS: Hypoplastic left heart syndrome, CPC: Cardiac progenitor cell, nCPCs: Neonatal CPCs, UCB: Umbilical cord blood, BNP: Brain natriuretic peptide UCB-MNCs: UCB-derived mononuclear cells

sustainability of stem cell therapies in HLHS. Addressing these challenges will be crucial for advancing stem cell therapy from experimental settings to widespread clinical use in HLHS patients.

CONCLUSIONS

HLHS, although less common than other CHD heart defects, remains a compelling field of study due to its significant mortality and morbidity rates, necessitating further exploration of effective treatment modalities. While palliative surgical interventions have extended the lifespan of affected individuals by creating an effective systemic right ventricular pump, persistent challenges in terms of quality of life and survival occur due to the development of RV overload-related dysfunction and failure. Several preclinical studies and clinical trials conducted using different types of stem cells and various administration routes in neonates and adults have demonstrated the efficacy of stem cells in regenerating damaged myocardial tissue and reducing heart failure. In addition, trials have observed that children may respond more favorably to stem cell therapy than adults, potentially increasing their chances of survival into adulthood. The shift of attention from a conventional stem-cell approach to a secretome-based, cell-free approach can further lead to a practically superior therapeutic option that will be easier to administer, require minimal immunosuppression, and is safer. The emerging technologies related to stem cells have the potential to bring about a revolutionary transformation in the treatment of various CHDs in the pediatric population, with a particular focus on HLHS. Nevertheless, we still need more extensive and prolonged clinical studies to identify the most suitable stem cell type, administration methods and sites, and effective treatment regimens and ensure both safety and effectiveness for clinical application to improve the quality of life in such patients.

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Conflicts of interest

There are no conflicts of interest.

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