Autologous stem cell therapy for hypoplastic left heart syndrome: Safety and feasibility of intraoperative intramyocardial injections

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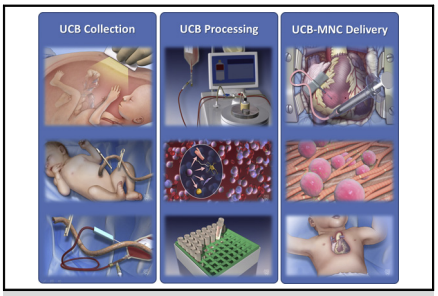
ABSTRACT

Objectives: Staged surgical palliation for hypoplastic left heart syndrome results in an increased workload on the right ventricle serving as the systemic ventricle. Concerns for cardiac dysfunction and long-term heart failure have generated interest in first-in-infant, cell-based therapies as an additional surgical treatment modality.

Methods: A phase 1 clinical trial was conducted to evaluate the safety and feasibility of direct intramyocardial injection of autologous umbilical cord blood-derived mononuclear cells in 10 infants with hypoplastic left heart syndrome at the time of stage II palliation.

Results: All 10 patients underwent successful stage II palliation and intramyocardial injection of umbilical cord blood-derived mononuclear cells. Operative mortality was 0%. There was a single adverse event related to cell delivery: An injection site epicardial bleed that required simple oversew. The cohort did not demonstrate any significant safety concerns over 6 months. Additionally, the treatment group did not demonstrate any reduction in cardiac function in the context of the study related intramyocardial injections of autologous cells.

Conclusions: This phase 2 clinical trial showed that delivering autologous umbilical cord blood-derived mononuclear cells directly into the right ventricular myocardium during planned stage II surgical palliation for hypoplastic left heart syndrome was safe and feasible. Secondary findings of preservation of baseline right ventricular function throughout follow-up and normalized growth rates support the design of a phase 2b follow-up trial. (J Thorac Cardiovasc Surg 2019;■:1-10)



The steps for successful cells injection in HLHS at the time of stage II palliation.

Central Message

Our phase 1 clinical trial showed that delivering autologous umbilical cord blood-derived mononuclear cells directly into the right ventricle myocardium during planned stage II surgical palliation was safe and feasible.

Perspective

Staged surgical palliation for HLHS results in an increased workload on the right ventricle serving as the systemic ventricle. Concerns for cardiac dysfunction and long-term heart failure have generated interest in first in-infant, cell-based therapies as an additional surgical treatment modality. Phase 1 clinical trial results of stem cell therapy for HLHS are presented.

See Commentary on page XXX.

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Abbreviations and Acronyms

BCPC = bidirectional cavopulmonary connection
HLHS = hypoplastic left heart syndrome
RVEF = visually estimated right ventricular ejection fraction
UCB-MNC = umbilical cord blood-derived mononuclear cells

Scanning this QR code will take you to the article title page to access supplementary information.

Hypoplastic left heart syndrome (HLHS) is a severe form of congenital cardiac disease accounting for 9% of children born with congenital cardiac anomalies.[1] HLHS involves a functionally single right ventricle with hypoplasia of the aorta and left heart structures resulting in the inability to maintain systemic cardiac output without intervention. If deemed surgical candidates, patients with HLHS will be offered 3 staged palliative operations, the first being the Norwood operation with aortic arch reconstruction, atrial septectomy, and pulmonary shunt creation, typically done within the first days to weeks of life. This is followed by a stage II bidirectional cavopulmonary connection (BCPC) operation at approximately age 4 months, and a stage III total cavopulmonary connection (ie, Fontan) operation at age 2 to 4 years. Because surgical mortality of the highest risk procedure at stage I has continued to improve,[2] the majority of families across the United States at most institutions are planning to achieve the Fontan circulation.[3] However, there remain populations within the United States and many other countries that still elect for termination of HLHS pregnancies given the requirement for extensive resources and risk of suboptimal outcomes that together fail to justify their commitment to managing this congenital heart disease with currently available surgical approaches.

Following successful surgical palliation there is indeed an increased workload on the single right ventricle that places increased risk of ventricular dysfunction and long-term cardiac failure. As this patient population continues to age, many may ultimately require cardiac transplantation. Although cell-based strategies in adults with cardiac failure have been reported, far less is known about the feasibility and safety in children with congenital heart disease.[4,5] Therefore, we developed an autologous cell-based clinical trial protocol for infants with HLHS that aimed to minimize potential risks associated with allogeneic cell sources and maximize the feasibility of autologous cell collection coupled with a cost-effective manufacturing process (Online Data Supplement). Because this was the first-in-child experience with intramyocardial delivery of stem cells, the inclusion and exclusion criteria were strictly defined to ensure that the most sensitive cohort to provide the most reliable safety readouts was studied. The objective of this longitudinal phase 2 study was to assess the safety and feasibility of direct intramyocardial injection of autologous umbilical cord blood-derived mononuclear cells (UCB-MNC) in infants with HLHS at the time of stage II surgical palliation.

METHODS

Study Design and Patient Population

This study was an open label, prospective, nonrandomized, phase 1 clinical trial (ClinicalTrials.gov identifier NCT01883076) designed to determine the safety and feasibility of UCB-MNC delivered into the right ventricular myocardium of subjects with HLHS at the time of a planned stage II Glenn surgical palliation (Figure 1). The study was conducted at Mayo Clinic (Rochester, Minn), with participating institutions including University of Oklahoma Health Sciences Center (Oklahoma City, Okla) and Children's Hospital of Philadelphia (Philadelphia, Pa). Subsequently, other centers were included under an extension of the phase 1 protocol (Children's Hospital Los Angeles, Los Angeles, Calif; Children's Minnesota, Minneapolis, Minn; and Children's Hospital Colorado, Aurora, Colo).

Prenatal diagnosis of HLHS was established by fetal echocardiography. Patients were enrolled in the ongoing Mayo Clinic Umbilical Cord Blood Collection Study for collection of cord blood cells for HLHS (ClinicalTrials.gov identifier NCT01856049) under an institutional review board approved protocol, following written informed consent. UCB was collected at the time of delivery and processed in the Human Cell Therapy Laboratory at Mayo Clinic from May 2012 through November 2016 and then ReGen Theranostics Inc (Rochester, Minn) from December 2016 through September 2018 to achieve release criteria of the manufactured autologous product. Postnatal transthoracic echocardiography confirmed the diagnosis of HLHS or HLHS variant. All enrolled patients underwent stage I palliation procedure.

Patients were assessed for participation in cell-based therapy clinical trial when they were considered candidates for stage II BCPC. Inclusion necessitated acceptable UCB-MNC collection and processing, the child being a candidate for stage II BCPC up to age 18 months, mother's serology was negative for human immunodeficiency virus, hepatitis B and C, and the child lacked study exclusion criteria as listed below. Exclusion criteria included cancer; severe chronic disease or extensive extracardiac syndromic features; history of dimethyl sulfoxide reaction in mother or child; child that has not completed preprocedural workup within 10 days before stage II surgery; child with currently active infection being treated with oral antibiotics; child with any of the following 15 days before stage II surgery that included urgent/unplanned procedure, cardiogenic shock, changes in medical therapy for pulmonary hypertension, change in arrhythmia medications, or infection treated with intravenous antibiotics; tricuspid repair and/or aortic arch repair at the time of stage II surgery; length of hospitalization of more than 60 days for previous stage I surgery; dietary modifications due to chronic and severe chylothorax; current or uncontrolled seizure or history of neurological injury with persistent deficit; severe tricuspid regurgitation before stage II surgery; history of mechanical circulatory support unless cardiac function was normal 10 days before stage II surgery; or other clinical concerns documented

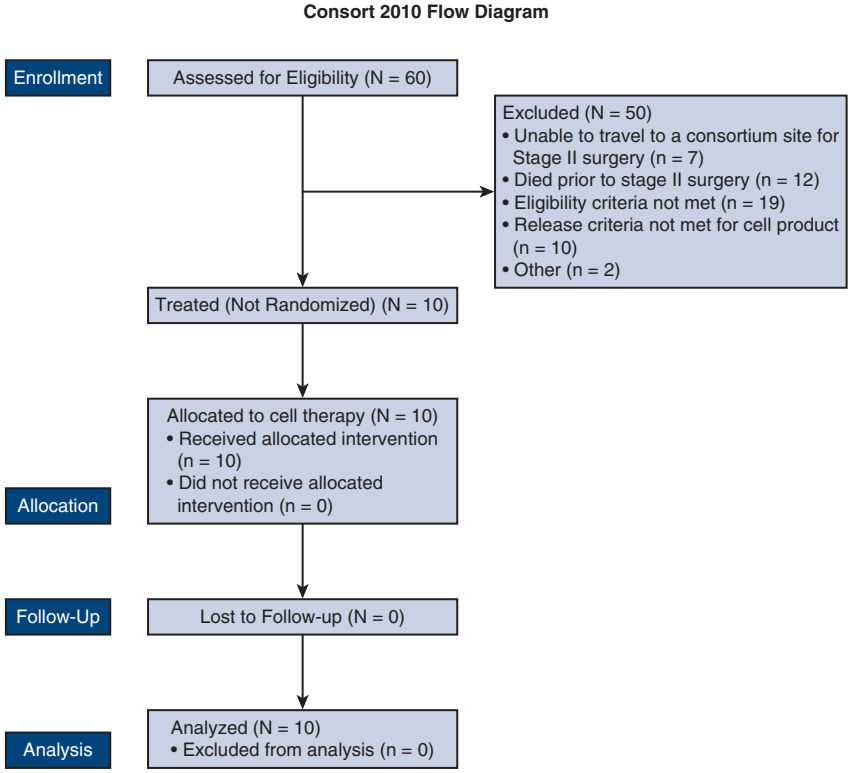

FIGURE 1. Study flow of the phase 2 clinical trial was designed to first secure autologous umbilical cord blood at the time of birth, consent for intraoperative cell injection at the time of scheduled stage II surgery, and then follow-up to 6 months in clinic to determine the safety and feasibility. The overview of the clinical participation notes the feasibility issues of this first-in-child study.

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by site investigator that would predict a high risk of severe complications during or after stage II surgery. The protocol utilized was monitored by both the Food and Drug Administration and local institutional review boards.

Because this study is first-in-child for cell-based therapy via an intraoperative intramyocardial delivery, the study team was required to have a minimum of 3-month follow-up data to be collected from the first child before enrolling and administering cell-based delivery to the second child, then likewise for the third child to be enrolled and treated. This staggered enrollment allowed adverse events to be reviewed before subsequent subject participation.

Preparation, Characterization, and Infusion of UCB-MNC

The autologous cell-based product was processed at Mayo Clinic Human Cell Therapy Laboratory and ReGen Theranostics, Inc, according to the procedures described in Investigational New Drug application 15343 (December 27, 2012; ClinicalTrials.gov identifier NCT01883076) that are specific and unique to the cardiac regenerative protocols that require a highly concentrated mononuclear cell product that is not equivalent to the manufacturing process used by traditional UCB banks, both private and public. The process of manufacturing this cardiac-specific product requires a unique UCB collection and cell preparation that allows a direct thaw and delivery at the time of cardiac surgery that is not possible with traditional umbilical cord collection protocols. The sponsor was responsible for reviewing the release criteria contained on the certificate of analysis from the manufacturer for each autologous product and determining acceptability of the release of the product to the investigational site for clinical use. Infants of serology positive mothers for hepatitis b, hepatitis C or human immunodeficiency virus were not eligible for participation in this initial study. At the time of stage II BCPC, the frozen autologous UCB-derived product was transported to the operating room and thawed per protocol once the patient was off cardiopulmonary bypass and after anticoagulation reversal. The product was administered via direct subepicardial intramyocardial injections of 0.1 mL per injection with 1 injection per kilogram body weight. Injections were spaced approximately 1 cm apart on the anterior surface of the right ventricle visually avoiding the coronary arteries. A 27-gauge needle was used, attached to a 1 mL syringe via flexible tubing that allowed the needle to be placed within the myocardial tissue parallel to the epicardial/luminal tissue plane sufficient to inject 0.1 mL volume with minimal leakage of the delivered product. The injections were performed slowly over at least 5 seconds and followed by a 20-second pause with the needle being left in place within the myocardium to avoid expulsion of cell product with premature withdrawal of the needle. Subjects received a target dose of 3 million cells per kilogram body weight of UCB-MNC via intramyocardial injection, with a range of 1 to 3 million cells per kilogram. This dose was chosen to be the maximal available dose of this autologous product based on average UCB collection across multiple sites that required at least 35 mL cord blood to yield a minimal product with 95% confidence.

Data Acquisition

Demographic, clinical, laboratory, and cardiac imaging data were acquired from the medical records. Adverse events and clinical outcomes were also acquired by reviewing the medical records. After cell-based therapy, all patients were monitored according to postsurgical standard of care. In addition, glucose and temperature monitoring were performed every 2 hours for the first 24 hours. C-reactive protein, creatine kinase MB, troponin T and N-terminal prohormone of brain natriuretic peptide were added to the standard blood draws at preoperative evaluation, at the

time of discharge, and at 6-month follow-up. Echocardiography and electrocardiography were performed preoperatively, at discharge and at the 1, 3, and 6-month follow-up visits. Visually estimated right ventricular ejection fraction (RVEF) was used for qualitative assessment of right ventricular function. Tricuspid annular plane systolic excursion, right ventricular index of myocardial performance, and right ventricular fractional area change were used for quantitative assessment of right ventricular function. Holter monitoring was performed preoperatively and at the 1-, 3-, and 6-month follow-up visits. In-clinic cardiac follow-up was completed at the 6-month visit. Adverse event assessment was performed at all encounters.

End Points

Feasibility analysis evaluated the ability to manufacture and release a cell-based product derived from the UCB samples meeting stringent pre-established criteria. The primary safety analysis encompassed adverse events after stage II BCPC surgery: death or any of the following adverse cardiac events, new or worsening: myocardial infarction, cardiac infection, heart failure, unexpected cardiovascular surgery, or sustained/symptomatic ventricular arrhythmias following the index time point (intramyocardial injection).

Statistical Analysis

Descriptive statistics included calculations of means and standard deviations for continuous variables and counts and percent for categorical variables. Patient weight quantiles were calculated using Centers for Disease Control and Prevention 2000 growth charts.[7] Adverse events are summarized as mean ± standard deviation of events per patient. RVEF and patient weight are plotted to illustrate patient progression through follow-up and percent weight change is scaled to 6 months. Statistical analysis was preformed using SAS software version 9.4 (SAS Institute Inc, Cary, NC) and R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Feasibility of Autologous UCB Collection and Processing

Between 2012 and 2016, a total of 35 UCB samples were processed at the Human Cell Therapy Laboratory at Mayo Clinic. Using equivalent processing protocols within an on-call and scalable process at ReGen Theranostics Inc starting at the beginning of 2017, an additional 74 UCB products were collected and manufactured. Together, a total of 109 UCB samples from HLHS newborns in 26 states and Canada have been processed with 80% success rate as defined by product meeting stringent pre-established release criteria. The failure to produce a suitable product was mostly linked to low cord blood collection volume that was <35 mL autologous cord blood (13 out of 22 samples; 60%) with the remaining failures randomly linked to inadequate labeling procedures, failure to follow collection procedures, low MNC percentage, and a single endotoxin positive assay. There were no positive bacterial cultures that would indicate contaminated collection or processing procedures in this initial cohort. Furthermore, the first 10 patients who received the intramyocardial injections of the experimental product all met release criteria with an average of 55 mL UCB at collection and an average of 20 hours from collection to cryopreservation to demonstrate the feasibility of this autologous product for this patient population within a centralized manufacturing process (Table 1).

Characteristics of the Patients

Between October 2013 and January 2018, 10 patients underwent successful intramyocardial injections of UCB-MNC at the time of stage II BCPC. Baseline characteristics at the time of stage II BCPC are shown in Table 2.

Patient Outcomes

All 10 patients underwent successful stage II BCPC and intramyocardial injection of UCB-MNC (Table 3). Operative mortality was 0%. Patient weight data demonstrated a trend of weight quantiles increasing over 6 months (Figure 2, *A*). Weight growth as percent increase scaled to 6 months is shown to be higher in this group (Figure 2, *B*). At 1-month follow-up, the natural history of

TABLE 1. Autologous umbilical cord blood derived product testing

Patient	Processing method	Time from collection to cryopreservation (h)	UCB collection volume (mL)	Pre-freeze viability (7-AAD) (%)	Prefreeze TNC ($\times 10^6$)	Prefreeze TNC concentration ($\times 10^6$ cells/mL)	TNC recovery (%)	Pre-freeze MNC (%)	MNC recovery (%)	Clinical vial (1.5 mL)	Endotoxin (EU/mL)	Sterility
1	Sepax DGBS	4.2	85	98	357	39.2	31	94	62	5	<1.00	Negative
2	Sepax DGBS	22.5	85	97	368	38.6	29	59	53	5	<1.00	Negative
3	Sepax DGBS	24	80	97	320	35.1	27	89	48	5	<1.00	Negative
4	Sepax DGBS	16.3	69	98	516	38.2	25	88	47	8	<0.5	Negative
5	Sepax DGBS	24.9	24	94	59	27.1	18	92	28	1	<0.5	Negative
6	Sepax DGBS	24.3	40	92	146	35.2	30	77	45	2	<0.1	Negative
7	Sepax DGBS	22.4	32	97	189	38.4	36	61	45	2	<0.1	Negative
8	Sepax DGBS	20.6	52	98	172	34.3	31	80	39	2	<0.1	Negative
9	Sepax DGBS	20.5	40	99	214	39.3	30	70	57	3	<0.1	Negative
10	Sepax DGBS	27.6	42	98	202	35.6	38	74	61	3	<0.1	Negative
Mean ± standard deviation		20.73 ± 6.55	55.0 ± 23.0	96.0 ± 2.15	254.0 ± 133.9	36.0 ± 3.68	29.0 ± 5.56	78.0 ± 12.5	48.0 ± 10.37	3.6 ± 2.12		

Sepax DGBS is manufactured by Biosafe America Inc, Houston, Tex. *UCB*, Umbilical cord blood; *7-AAD*, 7-aminoactinomycin D; *TNC*, total nucleated cells; *MNC*, mononuclear cells; *Sepax DGBS*, density gradient-based separation.

TABLE 2. Patient demographic characteristics at the time of bidirectional cavopulmonary connection (N = 10)

Characteristic	Result
Age at BCPC procedure (mo)	4.9 ± 1.4
Weight at BCPC procedure (kg)	6.2 ± 0.9
Weight quantile at Glenn*	28.1 ± 28.3
Male	8 (80)
HLHS	
MS/AS	2 (20)
MS/AA	2 (20)
MA/AS (DORV)	3 (30)
MA/AA	3 (30)
White	10 (100)
Hispanic	1 (10.0)

Values are presented as mean ± standard deviation or n (%). *BCPC*, Bidirectional cavopulmonary connection; *HLHS*, hypoplastic left heart syndrome; *MS*, mitral valve stenosis; *AS*, aortic valve stenosis; *AA*, aortic valve atresia; *MA*, mitral valve atresia; *DORV*, double outlet right ventricle. *Weight quantiles calculated using the Centers for Disease Control and Prevention 2000 growth charts[7] adjusting for patient's age and gender.

TABLE 3. Operative data for bidirectional cavopulmonary connection (N = 10)

Variable	Result
Additional procedure	
Pulmonary arterioplasty	1 (10)
Atrial septal defect enlargement	1 (10)
Cardiopulmonary bypass time (min)	56 (32-97)
Aortic crossclamp	
No. of patients	4 (40)
Time (min)	26 (25-42)
Deep hypothermic circulatory arrest	
No. of patients	1 (10)
Time (min)	25
Volume of UCB-MNC administered (mL)	0.6 (0.5-0.7)
Number of UCB-MNC injections	5.9 (1.2)
Length of hospital stay (d)	7 (6-11)

Values are presented as n (%) or median (range). *UCB-MNC*, Umbilical cord blood-derived mononuclear cells.

cardiac function as measured by RVEF on echocardiography had an undetectable change in RVEF (Table 4 and Figure 3). Lab data including N-terminal prohormone of brain natriuretic peptide, C-reactive protein, and blood glucose are shown in Table 5.

There was 1 late mortality that was determined not to be related to cell-based product or delivery. In brief, 3 months after stage II palliation and cell-based delivery, the patient with normal cardiac function underwent an elective surgical colostomy takedown that occurred secondary to necrotizing enterocolitis when hospitalized after stage II surgery. Postoperatively, the patient experienced respiratory collapse leading to a sequence of events that required emergency surgical procedures and further clinical complications. The patient was emergently listed for cardiac transplantation upon acute stabilization but was unable to receive a suitable organ before fatal multisystems failure.

Safety

Because of the 1 case of perioperative complications within the predefined 30-day window, the study was placed on clinical hold according to predefined stopping rules. Data and Safety Monitoring Board review recommended no changes or modifications to the protocol and concluded that there was no likely association of the clinical situation to either the cell product or the delivery of the cell product. The majority of all study-related adverse events were recorded from this first patient due to the complicated clinical course that followed. Beyond the adverse events from this single patient, there was no other significant adverse event. The treated patients had an average of 6.1 ± 3.9 minor adverse events. There was 1 adverse event directly related to cell product delivery. When undergoing UCB-MNC intramyocardial injections, 1 patient had an epicardial bleed that necessitated a simple suture oversew. There was no associated arrhythmia, hemodynamic instability or evidence of myocardial ischemia in the treated patients. Of 27 serious adverse events (50%) occurring in 5 patients, none were found to be related to the cell delivery or therapy and 12 of these events were attributed to the patient who died. With ongoing review by the Data and Safety Monitoring Board of all adverse events, there has been no recommendations for protocol changes or modifications due to safety concerns.

Comments

Children with HLHS need additional therapeutic strategies to strengthen the single right ventricle to sustain long-term pressure overload. Regenerative strategies are emerging as a focal point with the hope of offering therapeutic benefit to this patient cohort.[6] The phase 1 study presented herein represents the first study of intramyocardial cell-based therapy at the time of HLHS stage II palliation. Our central hypothesis is that direct delivery of autologous UCB-MNC into the right ventricular myocardium at the time of stage II palliation in HLHS patients will provide a feasible and cost-effective regenerative product for pediatric congenital heart disease while maximizing the long-term safety profile by using an autologous source. Of note, the use of autologous cells has been discounted by industry-sponsored clinical trials, in part, due to the presumed cost and feasibility limitations cited over the more profitable allogeneic products. Additionally, the best evidence available for autologous versus allogeneic bone marrow derived mesenchymal stem cells indicates 33% of adult patients receiving allogeneic products develop calculated panel reactive

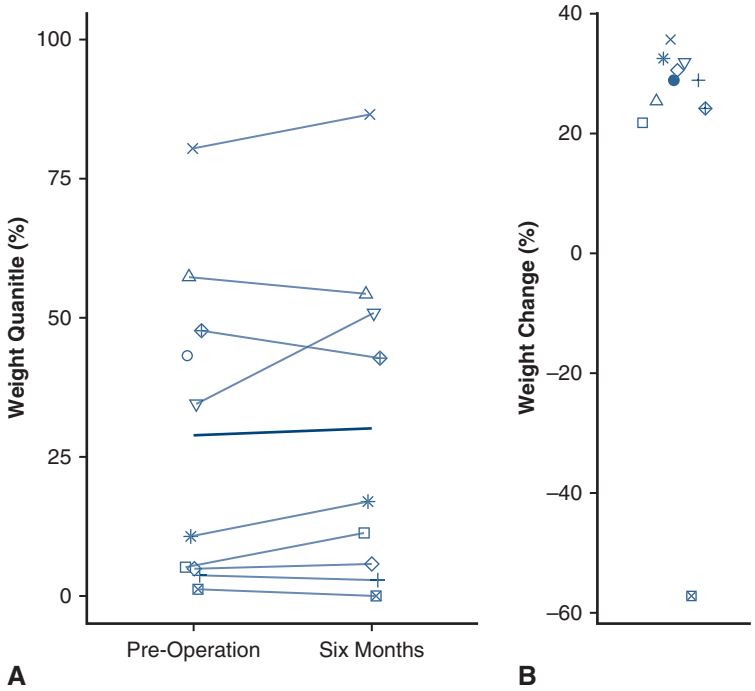

FIGURE 2. A, Plot of patient weight percentiles using the Centers for Disease Control and Prevention growth charts.[7] Individual patients are represented by unique symbols. One patient (*o*) did not survive until 6-mo follow-up visit. B, Plot of percent weight change preoperation to 6 mo scaled to 6 mo. Median is plotted.

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TABLE 4. Data from echocardiography (N = 10)

Variable	Result
Right ventricular index of myocardial performance	
Preoperation	0.43 ± 0.12
Discharge	0.47 ± 0.08
1-mo follow- up	0.40 ± 0.09
3-mo follow-up	0.42 ± 0.14
6-mo follow-up	0.21 ± 0.41
Right ventricular fractional area change (%)	
Preoperation	38.33 ± 9.42
Discharge	38 ± 6.83
1-mo follow-up	41.89 ± 8.68
3-mo follow-up	44.72 ± 8.76
6-mo follow-up	44.88 ± 6.58
Tricuspid annular plane systolic excursion (mm)	
Preoperation	8.78 ± 1.64
Discharge	6.2 ± 1.23
1-mo follow-up	7.67 ± 1.58
3-mo follow-up	8.41 ± 2.03
6-mo follow-up	8 ± 0.93
Visually estimated right ventricular ejection fraction (%)	
Preoperation	53.3 ± 7.23
Discharge	53 ± 7.89
1-mo follow-up	55.89 ± 8.12
3-mo follow-up	55.04 ± 5.81
6-mo follow-up	54.33 ± 6.18

Values are presented as mean ± standard deviation.

antibodies levels at either moderate or severe risk levels compared with 7% of adult patients receiving autologous products.[8] Thus, the undocumented and unknown long-term consequences of allogenic products in a pediatric population that is at high-risk of requiring cardiac transplantation compels the prioritization and comprehensive study of autologous cells in terms of safety, feasibility, and cost at this initial stage of product development.

Among the main end points for this phase 1 study was to address the feasibility of autologous UCB collection. The data herein demonstrates manufacturing capabilities to efficiently meet the needs of this patient population across the continental United States. Collection has been limited by the lack of full awareness of the protocol within prenatal care teams and the families during a highly complex time period of prenatal diagnosis of complex congenital heart disease. Furthermore, the volume of blood collection at the site of delivery directly influences the success rate of manufacturing a suitable product, which is a remarkable 96% success when >35 mL blood is collected. This volume can be achieved with focused training yet can also be inherently limited in the unexpected emergency settings that can happen at the time of any birth. The awareness of the availability of these studies within prenatal care teams can be optimized further with updated training and education to all prenatally diagnosed HLHS families by

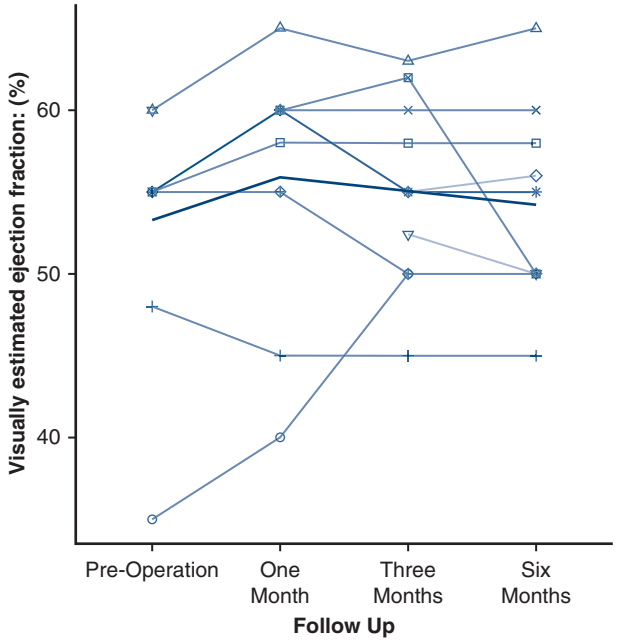

FIGURE 3. Plot of visually estimated right ventricular ejection fraction (RVEF) by echocardiography at each of the predefined time points according to the study protocol (each symbol represents a unique patient). Echocardiograms were collected at study sites according to predefined protocols and analyzed according to predefined imaging standards. As predicted according to inclusion/exclusion criteria, a majority of data was collected on patients with normal RVEF. The mean value of treated patients (*bold blue line*) does not indicate a drop in RVEF. Individual data points are shown separately.

TABLE 5. Laboratory data (N = 10)

Variable	Result
Glucose (mg/dL)	
Preoperation	94.6 ± 15.3
Discharge	96.6 ± 21.1
6-mo follow-up	80 ± 7.6
Troponin T (ng/mL)	
Preoperation	0.025 ± 0.04
Discharge	0.136 ± 0.21
6-mo follow-up	0.005 ± 0.01
Creatine kinase-MB	
Preoperation	6.7 ± 2.9
Discharge	3.2 ± 1.3
6-mo follow-up	6.2 ± 3.3
C-Reactive protein (mg/L)	
Preoperation	1.5 ± 2.5
Discharge	14.5 ± 10
6-mo follow-up	1.9 ± 3.5
N-Terminal prohormone of brain natriuretic peptide (pg/mL)	
Preoperation	5460 ± 8726
Discharge	2129 ± 2093
6-mo follow-up	661 ± 341

Values are presented as mean ± standard deviation.

partnering with patient-advocacy groups. There was 100% success rate of delivering the frozen products to requesting clinical sites at the time of stage II surgery using a standardized dry-shipper and dedicated manufacturing and surgical team. Therefore, the centralized manufacturing and logistics of cell-delivery were established to be highly reproducible and scalable to support a phase 2 study design.

Human UCB stem cells have been a focus in regenerative medicine with evidence of UCB-derived cells having the ability to differentiate into specialized cell types[9,10] and improve cardiac function in animal model systems with postinfarction of the left ventricle.[11-13] UCB-MNC injections into pressure overloaded right ventricles in small and large animal models have improved cardiac structure, reversal of pathologic gene expression profiles, and prevention of fibrotic changes.[14,15] Our preclinical data provide support for the paracrine hypothesis of UCB-MNC that could be driving tissue remodeling with angiogenesis in the right ventricular and proliferation of cardiac cell types.[16] Additionally, the paracrine effect of UCB-derived cell-based therapy in the preclinical setting may be directly stimulating endogenous cellular proliferation while shifting tissue remodeling pathways away from a fibrotic right ventricle response with chronic inflammation as UCB cells can express and secrete paracrine factors that can activate endogenous repair mechanism.[17] Others have utilized UCB-MNC in preclinical experimental approaches for myocardial repair and regeneration demonstrating improvement in the ejection fraction, wall motion, and cardiac contraction.[11-13,18-21] The collection of this preclinical body of work is further supported by recent success of UCB clinical studies in adult ischemic heart disease where elevated hepatic growth factor has been postulated as part of the mechanism of action.[22] Human UCB has also shown to enhance neonatal right ventricular function in an ovine model of right ventricle pressure overload.[23]

There is also a growing experience with cell-based regenerative cases and clinical studies for congenital heart disease. The initial experience with intracoronary delivery of bone marrow–derived mononuclear cells in 9 children with advanced heart failure was reported to show more than half improve clinically.[24] The largest experience reported in the Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease study was a randomized, controlled phase 2 trial, designed for efficacy evaluation of intracoronary cardiosphere-derived cell administration after stage II or III palliation for the treatment of children with single ventricle physiology compared with control participants receiving only a standard surgical procedure.[5] This study reported that intracoronary cardiosphere-derived cell infusion improved cardiac function (with reduced ventricular volumes and fibrosis), heart failure

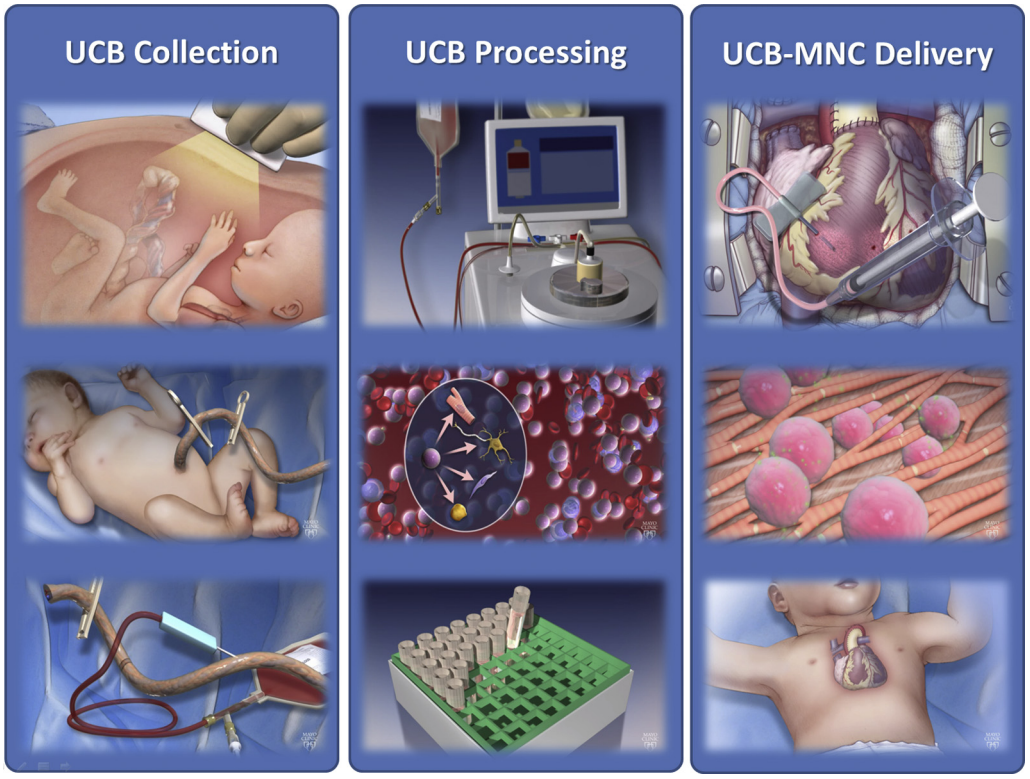

FIGURE 4. Diagnosis of hypoplastic left heart syndrome is established by fetal echocardiogram (*top left*). Umbilical cord blood (*UCB*) collection kit is sent to the family before birth and used to maximize collection volume (*middle and lower left*). UCB is collected by local hospital staff, packaged, and transported by dedicated courier to a specialized laboratory where the sample is processed to isolate the mononuclear cell product via Ficoll density gradient separation (*upper and middle center*). The final cell-based product is cryopreserved in sterile 2 mL cryovials with 10% dimethyl sulfoxide at a predetermined concentration and stored in vapor phase of liquid nitrogen (*lower center*). After the completion of superior cavopulmonary anastomosis, the UCB-derived mononuclear cells (*UCB-MNC*) are directly injected in the subepicardial myocardium (*top right*). These autologous cells are transiently residing and contributing only to the paracrine influence within the myocardial microenvironment (*middle right*) establishing the safety and feasibility of this novel experimental therapy (*bottom right*).

status, somatic growth, and health-related quality of life from baseline to 3- and 12-month end point analyses. Our initial case using UCB-MNC in this series of patients was reported in an infant that safely underwent cell delivery and was followed with improved cardiac function in the subsequent 3 months.[4] There is growing interest and worldwide effort to examine multiple cell types with the collective goal of establishing the evidence to support the safest and most cost-effective strategies.[25]

Herein, we report the outcomes of the initial 10 patients who safely received intramyocardial injection of autologous UCB-MNC at time of stage II palliation in HLHS patients (Figure 4). The quantitative assessment of right ventricular function by echocardiography in this initial cohort demonstrated no statistical differences from baseline to 6-month follow-up and suggests that cardiac function is not compromised in this cohort receiving the experimental product. Furthermore, patients who received the experimental cell-based product demonstrated preserved cardiac function within the focus of this study. A larger phase 2b study that includes patients for both treatment and control arms with normal and abnormal baseline RVEF will improve the sensitivity of revealing a potential benefit in patients with HLHS. The encouraging findings in this phase 1 study support the need for a phase 2b clinical trial using more sensitive imaging tools, such as cardiac magnetic resonance imaging, within a multicenter clinical trial study design to better estimate the magnitude of potential benefit.

Limitations

This phase 1 clinical trial with a limited number of patients characterized by mostly normal cardiac function as part of the inclusion criteria was not powered to assess efficacy of intramyocardial treatment of UCB-MNC. The limitations of a prospective control cohort to estimate the natural history of cardiac function will continue to be an issue given the inherent limitations of this rare disease population yet improving the feasibility of the study can be better addressed within a larger, multicenter clinical trial. Furthermore, nonstandard of care, preprocedural workup for families can be minimized in the phase 2b study to

ease recruitment in a control arm. Our anticipated phase 2b study has been designed to assess efficacy in up to 50 patients and includes a more reproducible imaging approach using cardiac magnetic resonance imaging that will be analyzed within a double-blinded imaging core to minimize any potential bias.

CONCLUSIONS

This phase1I trial delivering autologous UCB-MNC directly into the right ventricle myocardium during planned stage II surgical palliation was devoid of major safety concerns and is feasible with the dedicated team and infrastructure currently available within the Wanek HLHS Consortium. UCB collection and processing can achieve >90% success with >35 mL cord blood collection and thus establishes the feasibility of this autologous approach for a phase 2b study. The next step of a multicenter phase 2b study will be needed to further document the safety profile and to better define the potential for benefit of UCB-MNC during cardiac surgery.

Conflict of Interest Statement

Mayo Clinic has a financial interest in ReGen Theranostics Inc, with licensing and codevelopment agreements. Authors have nothing to disclose with regard to commercial support.

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000 Autologous stem cell therapy for hypoplastic left heart syndrome: Safety and feasibility of intraoperative intramyocardial injections

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Our phase 1 clinical trial showed that delivering autologous umbilical cord blood-derived mononuclear cells directly into the right ventricle myocardium during planned stage II surgical palliation was safe and feasible.