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# Autologous umbilical cord blood mononuclear cell therapy for hypoplastic left heart syndrome: a nonrandomized control trial of the efficacy and safety of intramyocardial injections

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## Abstract

**Background** Preliminary phase I clinical trial results revealed that autologous umbilical cord blood-derived mononuclear cells (UCB-MNCs) preserved right ventricular cardiac function. To establish the efficacy of intramyocardial injections of an autologous UCB-MNC product at the time of stage II palliation surgery in patients with hypoplastic left heart syndrome (HLHS).

**Methods** A phase IIb, multicenter, open-label, nonrandomized study was conducted. Ninety-five children (fifty treated and forty-five controls) with HLHS and its variants, a history of stage I palliation surgery, and planned stage II palliation surgery at less than thirteen months were enrolled. We assessed coprimary efficacy endpoints for changes in right ventricular cardiac function through fractional area changes and longitudinal and circumferential strain, both in the short term (three months) and long term (twelve months). Second, we assessed changes in biomarkers of cardiac injury. Safety endpoints included severe adverse events (SAEs), changes in overall health through vital signs, and cumulative hospitalization.

**Results** Assessment of our coprimary efficacy endpoints revealed an unfavorable change in longitudinal cardiac strain in the treatment group compared with an improvement in strain in the control group (unadjusted $p=.032$) in the short term. No differences were observed between the groups in terms of other coprimary efficacy endpoints in the short or long term. A secondary assessment of biomarkers of cardiac injury revealed higher troponin T levels in the treatment group at three and six hours postsurgery. Regarding safety, no deaths related to the administered product or delivery procedure were reported. The treatment group presented a greater incidence (20%) of at least

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one SAE than the control group at three months ($p=.048$). Additionally, no statistically significant differences were found for the other safety endpoints.

**Conclusion** Intramyocardial injections of autologous UCB-MNC products into the right ventricular myocardium during stage II palliation surgery failed to enhance cardiac function in patients with hypoplastic left heart syndrome.

**Registered on ClinicalTrials.gov** Registered on ClinicalTrials.gov (NCT03779711) on 12/04/2018; URL: https://clinicaltrials.gov/ct2/show/NCT0377971.

**Novelty and significance**

*What is known?*

• To date, no prior efficacy studies have examined the use of autologous umbilical cord blood-derived mononuclear cells (UCB-MNCs) in patients with hypoplastic left heart syndrome following stage palliation surgeries.

• A preliminary phase I clinical trial noted that early follow-up (six months) after the administration of autologous UCB-MNC enhanced right ventricular cardiac function relative to that of controls, alongside greater weight percentiles [19].

*What new information does this article contribute?*

• Largest worldwide stem cell phase IIb clinical trial on congenital heart disease, specifically investigating the efficacy and safety of an autologous UCB-MNC product in patients with hypoplastic left heart syndrome.

• The autologous UCB-MNC product depicted a favorable safety profile, with no severe adverse events or fatalities related to the product or product administration.

• Despite the favorable safety profile observed, intramyocardial injections of the autologous UCB-MNC product did not yield improvements in cardiac function in the short or long term.

The preservation of right ventricular cardiac function remains a critical challenge in HLHS, as evidenced by several studies documenting a decline in right ventricular contractility over time at various stages of surgical palliation. The use of stem cells is effective in regenerating, remodeling, and renewing injured myocardium. Although preliminary clinical trials reported improved cardiac function following the administration of autologous UCB-MNC in HLHS patients, this study failed to demonstrate the preservation of cardiac function while maintaining safety. The multicenter nature and magnitude of this dataset are substantial and should not be understated; however, we must also acknowledge the limitations of our study, underscoring the need for further research to address them and optimize the therapeutic potential of UCB-MNC therapy for HLHS. We are committed to continuing our efforts to provide more bases and insights into the mechanisms and a better understanding of regenerative medicine to find solutions for congenital heart disease.

**Keywords** Hypoplastic left heart syndrome, Single ventricle, Umbilical cord blood stem cell transplantation, Mononuclear cells, Stem cell, Regenerative therapy

## Background

Regenerative medicine emerged in the early 21st century as a promising approach to address the challenges faced by conventional medicine in effectively treating diseases and conditions that have shown disheartening outcomes. It has expanded the knowledge and provided a new perspective on the mechanisms of these diseases. This field encompasses a thorough understanding of stem cell biology and other basic sciences, such as biochemistry, genetics, immunology, and computer sciences. Its primary goal is to restore the functionality of weakened or damaged cells through a comprehensive process that involves manipulating healthy progenitor cells or undifferentiated stem cells, thereby facilitating the restoration and optimization of their inherent capabilities.

Congenital heart disease (CHD) has a prevalence of 8.1 per 1,000 live births [1]. HLHS, the most common form of single-ventricle CHD, has a prevalence of 2–3 per 10,000 live births and the highest mortality when treatment is not offered in a timely manner at birth [1–4]. HLHS is associated with impaired heart function due to the underdevelopment of left heart structures (5). The management of this condition comprises multistage palliative surgeries, which start at birth, continue at 4–6 months of age, and end with Fontan surgery at approximately 18–48 months of age [6, 7]. Most newborns currently undergoing surgery for HLHS survive, with approximately two-thirds alive after stage III palliation; within this group, approximately 80% continue to survive for up to 20 years after that [8]. Despite improving survival rates, these children still face long-term risks attributed to multiorgan system failure, partly due to right ventricular dysfunction, resulting in an 8% transplant risk within 20 years [9, 10].

In recent years, numerous clinical trials have emerged as alternative approaches to improve cardiac function in children with CHD, particularly those with right ventricle physiology. Clinical trials involving CHD in

children involve several factors to consider, including donor selection (allogeneic vs. autologous), the type of tissue (cardiac, bone marrow, or umbilical cord blood), the administration route (intracoronary, intramyocardial, epicardial, or intravenous), and the timing of administration during multistage surgical palliation [11].

In 2015, a team from Okayama University in Japan published the first phase I and later phase II clinical trials investigating transcoronary infusion of cardiac progenitor cells in patients with single-ventricle physiology [12]. Other trials include phase I/IIb clinical trials in which allogeneic human mesenchymal stem cell injections were used during stage I palliation surgery in patients with hypoplastic left hearts [13].

However, it is essential to acknowledge that the development of stem-cell clinical trials for CHD in children is years behind compared with the extensive experience from numerous trials in adult heart disease [14–17]. Our team focused on stem cell therapies involving autologous bone marrow and umbilical cord blood-derived mononuclear cells (UCB-MNCs). In 2019, we published the first groundbreaking phase I clinical trial involving single-dose intramyocardial injections of autologous UCB-MNC products in ten children with HLHS during the bidirectional Glenn shunt procedure (stage II palliation surgery). This trial successfully demonstrated the safety and feasibility of the autologous UCB-MNC product. In addition, the preservation of baseline right ventricular cardiac function and the normalized growth rates in the follow-up period motivated us to substantiate this therapy's evidence and benefits further [18]. Hence, we conducted this longitudinal phase IIb clinical trial to assess the safety and effectiveness of the autologous UCB-MNC product through intramyocardial injections in a group of patients with HLHS compared with control subjects.

## Methods

### Study design and participants

This study is a multicenter, prospective, open-label, nonrandomized, observational phase IIb clinical trial designed to determine the safety and efficacy of a UCB-MNC product delivered into the right ventricular myocardium of patients with HLHS and its variants at the time of stage II palliation surgery *(*Fig. 1*)*. This study focused primarily on short- and long-term right ventricular cardiac function. Second, cardiac injury was measured through cardiac biomarkers [troponin T and B-type natriuretic peptide (BNP)], severe adverse events (SAEs), cumulative days of hospitalization, vital signs, and parents' perceptions of their child's overall health and developmental ability. This study was conducted at Mayo Clinic (Rochester, MN), Children's Hospital Colorado (Aurora, CO), Children's Hospital of Alabama (Birmingham, AL), Cincinnati Children's Hospital Medical Center (Cincinnati, OH), Ochsner Medical Center Jefferson (Jefferson, LA), Children's Hospital Los Angeles (Los Angeles, CA), Children's Hospital and Clinics of Minnesota (Minneapolis, MN), Oklahoma Children's Hospital, University of Oklahoma (Oklahoma City, OK), and Children's Hospital of Philadelphia (Philadelphia, PA).

### Eligibility and enrollment

Before enrollment, expecting parent(s) with a prenatal diagnosis of HLHS or HLHS variants by fetal echocardiogram were enrolled in the ongoing Mayo Clinic umbilical cord blood (UCB) study for collection of UCB cells for HLHS (ClinicalTrials.gov identifier NCT01856049)

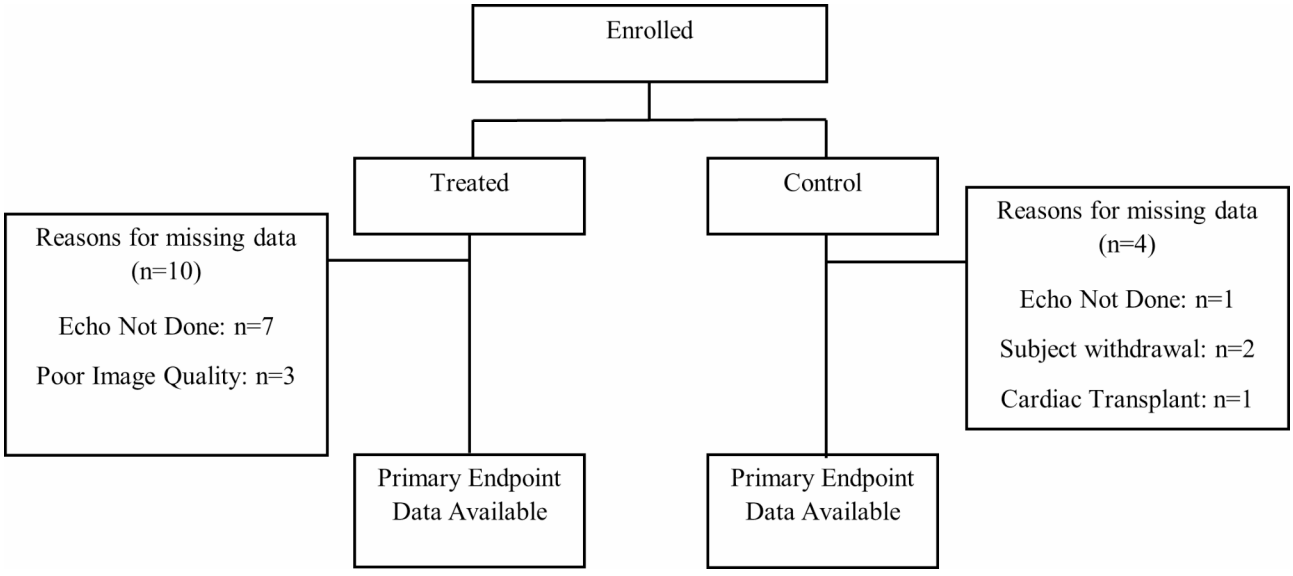


**Fig. 1** Consort flow diagram showing the number of enrolled subjects, subject allocation into treatment groups, and primary endpoint data available

under an institutional review board-approved protocol. Our study population comprised fifty subjects enrolled in the treatment group and forty-five controls. Both groups met identical study eligibility criteria, study tests, and procedures, except for the requirements related to the acceptability of the UCB-MNC product and the sensitivity of the dimethyl sulfoxide (DMSO) reaction (treatment group only). The inclusion criteria included a diagnosis of HLHS or an HLHS variant with single right ventricular-dependent CHD, a history of prior stage I palliation surgery, being scheduled for stage II palliation surgery within less than thirteen months of age after stage I surgery, and having participated in the UCB collection protocol with the autologous UCB-MNC product that was deemed acceptable for clinical use (treatment group only). The exclusion criteria included a history of DMSO, parent(s) and legal guardian(s) unwilling to have their child participate, severe chronic diseases, extensive extracardiac syndromic features, a known history of cancer, and any of the following: complications of congenital heart disease, any condition requiring urgent or unplanned interventional procedures within 15 days before stage II palliation surgery.

The study was approved by the Institutional Review Boards (IRBs) of the corresponding site institutions, which are as follows: Colorado Multiple IRB; Children's Hospital Los Angeles IRB; Children's Minnesota IRB; The Children's Hospital of Philadelphia Research Institute IRB; Cincinnati Children's Hospital IRB; Ochsner Clinic Foundation IRB; The University of Oklahoma - IRB for the Protection of Human Subjects; The University of Alabama at Birmingham - Office of the IRB for Human Written informed consent was obtained from all parents and legal guardian(s) of the subjects meeting the eligibility criteria. After providing consent, a screening phase was conducted that included a preoperative baseline evaluation: clinical evaluation, laboratory and imaging tests, and questionnaires, followed by stage II palliation surgery, which included enrollment. Subjects were actively followed up after surgery via in-clinic visits and telephone follow-ups. The protocol utilized was monitored by both the Food and Drug Administration (FDA) and the IRBs. One of our authors had full access to all the data in the study and took responsibility for its integrity and data analysis.

### Manufacturing, storage, transportation, and administration of the UCB-MNC product

The autologous UCB used to manufacture the cell-based product was collected under the UCB collection study protocol at birth and shipped to ReGen Theranostics, Inc. (Rochester, MN) for manufacturing. Manufacturing this cardiac-specific product requires a unique UCB collection and cell preparation that is different from the traditional UCB collection and processing protocols that allow direct thawing and delivery at surgery. The sponsor was responsible for reviewing the release criteria on the certificate of analysis from the manufacturer for each autologous product and determining the acceptability of the product's release to the investigational site for clinical use. During stage II palliation surgery, the frozen autologous UCB-derived product was transported to the operating room and thawed per protocol once the subject was off cardiopulmonary bypass and after anticoagulation reversal.

#### *Manufacturing*

Mononuclear cell enrichment was performed via Ficoll-based density gradient purification using the Sepax 2 Cell Processing System (GE Healthcare). The Sepax 2 is a closed system for cell separation, washing and concentration. Following a final centrifugation step, the UCB-MNCS were suspended in CryoStor10, a commercially available dimethyl sulfoxide-based cryopreservation solution, at concentrations ranging from 12 to 42 million cells per mL. Sterile cryovials were manually filled after at processing. The number of vials in each batch is variable and depends on cell yield. Each vial is labelled with product specific identification and a unique vial specific identifier. After processing, each product undergoes product characterization testing to meet all pre-defined release criteria. Characterization and process markers captured are hours from collection to finish, CD45, CD34, cell count, MNC total, MNC%, TNC, and viability.

#### *Storage and transportation*

The cells were stored under Good Manufacturing Practice (GMP) standards at the manufacturing facility. The products were frozen using a controlled-rate freezing process and subsequently transferred to liquid nitrogen (vapor phase) for long-term storage. For transportation, the cells were shipped in a liquid nitrogen dry shipper to the administration hospital by sponsor staff at the time of cell delivery, with no product storage at the clinical site. At the time of cell delivery, the cells are thawed by hand and immediately loaded into the delivery device with no additional manipulation. To safeguard against contamination or degradation, product delivery begins within 30 min of thawing. The procedure was canceled if a backup vial was unavailable in the surgical suite.

#### *Product dose and administration*

The subjects in the treatment group received a target dose of 1–3 million total nucleated cells per kg of body weight of the autologous UCB-MNC product via intramyocardial injections. A 27-gauge needle attached to a 1 mL syringe was used to administer the product via direct subepicardial injections of 0.1 mL per 1 kg of body weight

to reach the target dose. Injections were given slowly over at least 5 s, followed by a 20-second pause with the needle left in place to maximize cell retention within the myocardium. The injections were spaced approximately 1 cm apart across the free wall of the right ventricle. The injection sites were documented on a diagram to record the approximate location of the injection sites relative to the coronary vasculature and filed within each subject's study file.

Cell dosing is based on subject weight, and the volume for injection to achieve the desired dose is calculated at the time of cell delivery. The target dose range was established according to experience with our preclinical studies based on variability with cell recovery in the animal models, and was used as the acceptable range for our initial phase I and the current phase II study.

The rationale for intramyocardial delivery through direct subepicardial injections was chosen based on minimizing the risk of cell delivery for these single ventricle patients. Because these patients are required to undergo open chest cardiac surgery, the epicardial surface is routinely exposed, and intramyocardial delivery at the time of surgery as an "add-on" was determined to be less invasive and less risky compared to intracoronary infusion, which would require a separate catheter-based procedure and its associated ionizing radiation. Intramyocardial injections of UCB-MNC and subsequent follow-up were conducted in preclinical studies, which confirmed the feasibility, safety, and efficacy of cell delivery.

### Data acquisition

After consent and before stage II palliation surgery, demographic, baseline clinical, and laboratory data were collected, as were baseline quantitative measurements of cardiac function data with transthoracic echocardiography (TTE). Operative and postoperative data were also collected, including physical examination, daily vitals, and telemetry monitoring to identify arrhythmias and other adverse events; likewise, at one, three, and twelve months after surgery, physical examination, blood workup, and TTE were performed. The Infant Toddler Quality of Life Questionnaire-97 (ITQOL-97) and the Ages and Stages Questionnaire-3 (ASQ-3) scores were also recorded at baseline and the twelve-month follow-up. The collected data were filed into applicable electronic case report forms (eCRFs) in the electronic data capture system.

### Echocardiography data

To measure our coprimary endpoints, we planned for each of the ninety-five subjects to undergo a TTE at baseline,at discharge, and at three and twelve months after surgery. Right ventricular cardiac function was measured through fractional area change (FAC) and longitudinal and circumferential strain. This study used a blinded imaging core laboratory to evaluate the echocardiography images. The imaging core lab comprises Mayo Clinic staff not affiliated with this clinical trial.

### Endpoints

Our coprimary efficacy endpoints assessed right ventricular cardiac function changes from baseline to three- and twelve-months following stage II palliation surgery. This assessment was conducted by measuring FAC and circumferential and longitudinal strain. The secondary endpoints assessed changes in biomarkers of cardiac injury (troponin-T, BNP) at three and six hours post surgery. Additionally, the safety endpoints included SAEs; changes in overall health, measured by vital signs (weight, heart rate, and oxygen saturation) at three and twelve months; and the cumulative incidence of hospitalization at one and three months. Finally, we measured developmental abilities at twelve months with the ITQOL-97 and ASQ-3 assessment tools following stage II palliation surgery.

### Statistical analysis

Continuous data are presented as medians (IQRs), and categorical data are presented as counts (%). The Kruskal–Wallis or Wilcoxon rank sum test was used for between-group comparisons of continuous data. Categorical data were compared between groups via the chi-square test. Analysis of covariance (ANCOVA) models were used to test multiple outcomes between groups. For all ANCOVA models, we calculated the change from baseline, tested the computed change between groups, and adjusted for the baseline value of the dependent variable. Additional covariates were added in a multivariable sensitivity analysis to account for potential effect modifiers. The manuscript presents the marginal means of the ANCOVA models as LS means. Spearman correlation was used to assess the relationship between characterization and process markers and the primary outcomes. The primary and secondary endpoints were defined a priori, with additional comparisons added to better describe the sample, baseline status, and group differences in this work. An alpha of 0.05 was prespecified, and coprimary endpoints were adjusted post hoc to preserve alpha using the Bonferroni adjustment. No other adjustments were made for multiple testing outside the coprimary endpoints, and these results should be considered exploratory and not inferential. All analyses were performed via SAS Software (version 9.4), SAS Institute, Inc.

## Results

Between June 2019 and September 2021, ninety-five subjects were enrolled. Fifty subjects were successfully enrolled in the treatment group, while forty-five were

enrolled as controls. Our primary analysis included an echocardiographic evaluation of right ventricular cardiac function; unfortunately, owing to the lack of echocardiography data ($n=8$), poor image quality ($n=3$), subject withdrawal ($n=2$), and cardiac transplant ($n=1$), primary endpoint data could only be obtained for eighty-one subjects, forty in the treatment group and forty-one in the control group (Fig. 1).

### Baseline

Demographics, vital signs, blood workup, and cardiac function data are summarized in Table 1. Both groups were predominantly composed of white males, with an even more pronounced representation of the treatment group, and had comparable anthropometric measurements and vital signs. The subjects in the treatment group were younger, with a median age of 4.7 months ([IQR, 4.5, 5.1] compared with 5.2 months [IQR, 4.4, 6.2] in the control group, ($p=.048$)). There were no significant differences between the groups regarding baseline cardiac function, cardiac biomarkers, or blood workup.

Stage I palliation surgery with and without a valved RV-PA shunt accounted for 78.0% (44.0% nonvalved, 34.0% valved) of stage I surgeries in the treatment group and 82.2% (71.1% nonvalved, 11.1% valved) in the control group ($p=.049$). For stage II palliation surgery, the most common surgery type was the Glenn procedure, accounting for 88.0% of the patients in the treatment group and 93.3% in the control group. The Hemi-Fontan procedure accounted for 12.0% and 6.7%, of the two groups, respectively. During this procedure, subjects treated with the UCB-MNC product had a shorter cardiopulmonary bypass time, with a median duration of 64.0 min ([IQR, 38.0, 91.0] compared with 83.0 min [IQR, 51.0, 113.5] in the control group, $p=.024$]. No complications were reported during surgery.

The baseline assessment of parental and legal guardians' perceptions of their children's overall health and developmental abilities with the ITQOL-97 and ASQ-3 assessment tools revealed better scores for the gross motor and personal social domains in the ASQ-3 tool in the subjects in the treatment group. In contrast, no differences were reported in overall health with the ITQOL-97 (See Supplementary Tables S1 and S2, Additional file 1).

### Co-primary endpoints

#### *Right ventricular cardiac function at three (short-term) and twelve months (long-term)*

The ANCOVA model did not reveal any statistically significant difference in FAC or circumferential strain in the short or long term. However, longitudinal strain demonstrated an unfavorable change in the treatment group in the short term ($p=.032$, unadjusted; $p=.19$, adjusted). The LS mean estimate was 0.539 (95% CI -0.607–1.686) for the treatment group and – 1.240 (95% CI -2.387 - -0.094) for the control group (Table 2). The difference in longitudinal strain was not observed in the long term ($p=.254$, unadjusted; $p=1.00$, adjusted).

To account for potential effect modifiers, we adjusted the ANCOVA model for baseline stage I surgery type, tricuspid regurgitation, and cardiopulmonary bypass time (Table 3). This adjustment proved that subjects receiving the UCB-MNC product experienced an unfavorable change in longitudinal strain in the short term ($p=.009$). The LS mean estimate for the UCB-MNC group was 0.646 (95% CI -0.493–1.786), whereas the LS mean estimate was – 1.555 (95% CI -2.695 - -0.415) for the control group. Like the initial analysis, no differences were observed in the longitudinal strain at twelve months or in the FAC or circumferential strain at three and twelve months.

### Secondary endpoints

#### *Cardiac injury*

Compared with those at baseline, troponin T levels were higher at three and six hours after surgery in the treatment group [(0.790 vs. 0.595, $p=.037$ at 3 h) and (0.700 vs. 0.539, $p=.032$ at 6 h)]. While the levels remained elevated in both groups at three months (0.417 vs. 0.425), there was no significant difference between them ($p=.994$). By twelve months, the troponin levels had decreased in both groups (-0.024 vs. -0.025, $p=.147$), as shown in Table 3.

BNP levels were predictably elevated before surgery and declined as anticipated during follow-ups at three and twelve months. However, no significant differences were found between the groups (See Supplementary Table S3, Additional file 1). Spearman correlation analysis did not reveal any association between characterization and process markers and the co-primary endpoints in the treated group.

### Safety endpoints

#### *Adverse events*

The subjects in the treatment group presented a 20% greater cumulative incidence of at least one severe adverse event (SAE) at three months (58.0% vs. 37.8%, $p=.048$) of enrollment. The difference was not maintained at one year of enrollment (68.0% vs. 53.3%, $p=.143$)—Table 4. Among those with at least one SAE, the median number of SAEs observed within one and three months of enrollment was 2.0 for subjects in the treatment group and 1.0 for those in the control group. Through one year of enrollment, the median SAEs remained at 2.0 for the treatment group and increased to 1.5 for the control group (Table 5). Figure 2 illustrates the incidence of adverse events at one year of enrollment by system organ in each group.

**Table 1** Demographics, baseline clinical characteristics, cardiac function, cardiac biomarkers, blood workup, and type of stage I and stage II palliation surgery, including postoperative troponins and blood pressure measurement

| | Treatment group ($n=50$) | Control group ($n=45$) | *P*-value |
|---|---|---|---|
| **Age, months** | | | 0.048* |
| Median (Q1, Q3) | 4.7 (4.5, 5.1) | 5.2 (4.4, 6.2) | |
| **Gender** | | | **0.019**† |
| Female | 11 (22.0%) | 20 (44.4%) | |
| Male | 39 (78.0%) | 25 (55.6%) | |
| **Race** | | | 0.273† |
| White | 36 (72.0%) | 29 (64.4%) | |
| Black/African American | 3 (6.0%) | 3 (6.7%) | |
| Asian | 3 (6.0%) | 1 (2.2%) | |
| More than one | 3 (6.0%) | 2 (4.4%) | |
| Unknown, none or no reported | 5 (10.0%) | 10 (22.2%) | |
| **Weight**, **kg** | | | 0.538* |
| Median (Q1, Q3) | 6.0 (5.6, 6.7) | 6.1 (5.7, 6.6) | |
| **Height**, **cm** | | | 0.552* |
| Median (Q1, Q3) | 62.0 (60.0, 65.0) | 61.0 (59.0, 64.0) | |
| **Heart Rate/Pulse**, **bpm** | | | 0.232* |
| Median (Q1, Q3) | 123.5 (115, 137) | 122 (110, 132) | |
| **SBP**, **mmHg** Median (Q1, Q3) | 95 (74.0, 131.0) | 94.5 (74.0, 173.0) | 0.544* |
| **DBP**, **mmHg** Median (Q1, Q3) | 50.5 (28.0, 89.0) | 53.0 (29.0, 95.0) | 0.787* |
| **SBP at discharge**, **mmHg** Median (Q1, Q3) | 96.5 (69.0, 138.0) | 94.0 (69.0, 135.0) | 0.069* |
| **DBP at discharge**, **mmHg** Median (Q1, Q3) | 50.0 (37.0, 89.0) | 52.0 (34.0, 81.0) | 0.222* |
| **SPO2 (%)** | | | 0.590* |
| Median (Q1, Q3) | 80.5 (77, 84) | 80.0 (78, 84) | |
| **Cardiac function as assessed by echocardiography** | | | |
| **FAC**, **% (n)** | 48 | 45 | 0.963* |
| Median (Q1, Q3) | 41.7 (37.1, 44.9) | 41.9 (38.1, 45.0) | |
| **Circumferential Strain**, **% (n)** | 43 | 39 | 0.463* |
| Median (Q1, Q3) | -15.3 (-17.7, -11.9) | -16.4 (-18.9, -12.5) | |
| **Longitudinal Strain**, **% (n)** | 48 | 45 | 0.137* |
| Median (Q1, Q3) | -14.5 (-17.0, -12.4) | -15.9 (-18.0, -13.4) | |
| **Cardiac biomarkers** | | | |
| **Troponin-T**, **ng/mL (n)** | 42 | 41 | 0.376* |
| Median (Q1, Q3) | 0.025 (0.018, 0.039) | 0.024 (0.013, 0.038) | |
| **Troponin-T**, **3 h post-surgery**, **ng/mL (n)** Median (Q1, Q3) | 42 0.797 (0.136, 2.322) | 40 0.643 (0.155, 1.424) | 0.131* |
| **Troponin-T**, **6 h post-surgery**, **ng/mL (n)** Median (Q1, Q3) | 46 0.666 (0.000, 1.763) | 39 0.583 (0.015) | 0.255* |
| **NT-Pro-BNP**, **pg/mL (n)** | 48 | 43 | 0.450* |
| Median (Q1, Q3) | 1296.0 (687.5, 3066.0) | 1200.0 (855.0, 2196.0) | |
| **CRP**, **mg/L (n)** | 49 | 42 | 0.105* |
| Median (Q1, Q3) | 5.0 (2.9, 5.0) | 5.0 (2.5, 6.0) | |
| **Liver/Renal Function** | | | |
| **ALT**, **U/L (n)** | 48 | 42 | 0.420* |
| Median (Q1, Q3) | 25.0 (19.1, 34.0) | 22.0 (17.0, 37.0) | |
| **AST**, **U/L (n)** | 48 | 42 | 0.938* |
| Median (Q1, Q3) | 43.0 (36.0, 53.0) | 40.5 (36.0, 56.0) | |
| **Alk Phosphatase**, **U/L (n)** | 48 | 42 | 0.087* |
| Median (Q1, Q3) | 269.5 (222.0, 333.5) | 237.0 (197.0, 309.0) | |

**Table 1** (continued)

| | Treatment group ($n=50$) | Control group ($n=45$) | *P*-value |
|---|---|---|---|
| **Bilirubin, mg/dL (n)** | 48 | 42 | 0.948* |
| Median (Q1, Q3) | 0.4 (0.3, 0.5) | 0.4 (0.3, 0.6) | |
| **Albumin, g/dL (n)** | 48 | 43 | 0.529* |
| Median (Q1, Q3) | 3.5 (3.2, 4.2) | 3.5 (3.1, 4.1) | |
| **Total proteins, g/dL (n)** | 48 | 41 | 0.211* |
| Median (Q1, Q3) | 5.6 (5.3, 6.1) | 5.4 (5.0, 5.9) | |
| **Creatinine, mg/dL (n)** | 48 | 43 | 0.049* |
| Median (Q1, Q3) | 0.2 (0.2, 0.3) | 0.2 (0.2, 0.2) | |
| **BUN, mg/dL (n)** | 48 | 43 | 0.503* |
| Median (Q1, Q3) | 11.0 (8.0, 15.0) | 12.0 (8.0, 16.0) | |
| **CBC w/Differential** | | | |
| **Hemoglobin, g/dL (n)** | 49 | 45 | 0.365* |
| Median (Q1, Q3) | 15.1 (13.9, 16.1) | 15.6 (14.2, 16.3) | |
| **Hematocrit, % (n)** | 49 | 45 | 0.386* |
| Median (Q1, Q3) | 46.1 (42.9, 50.0) | 46.6 (43.9, 50.6) | |
| **WBCs, (n)** | 49 | 45 | 0.671* |
| Median (Q1, Q3) | 7.2 (5.7, 8.6) | 7.2 (5.8, 8.9) | |
| **Platelets, (n)** | 49 | 45 | 0.379* |
| Median (Q1, Q3) | 332.0 (278.0, 390.0) | 303.0 (264.0, 360.0) | |
| **Lymphocytes, (n)** | 47 | 45 | 0.046* |
| Median (Q1, Q3) | 3.5 (2.4, 5.6) | 3.1 (2.1, 4.1) | |
| **Type of stage I and stage II palliation surgery** | | | |
| **Stage I surgery type** | | | **0.049**† |
| Norwood procedure with mBT shunt | 8 (16.0%) | 5 (11.1%) | |
| Norwood procedure with nonvalved RV- PA shunt | 22 (44.0%) | 32 (71.1%) | |
| Norwood procedure with valved RV-PA shunt | 17 (34.0%) | 5 (11.1%) | |
| Hybrid Procedure | 2 (4.0%) | 1 (2.2%) | |
| Other (specify) | 1 (2.0%) | 2 (4.4%) | |
| **Stage II surgery type** | | | 0.375† |
| Glenn procedure | 44 (88.0%) | 42 (93.3%) | |
| Hemi-Fontan | 6 (12.0%) | 3 (6.7%) | |
| **Stage II Surgery Details** | | | |
| **Cardio-pulmonary bypass time, min (n)** | 48 | 44 | **0.024*** |
| Median (Q1, Q3) | 64.0 (38.0, 91.0) | 83.0 (51.0, 113.5) | |
| **Aortic cross-clamp time, min (n)** | 19 | 22 | 0.732* |
| Median (Q1, Q3) | 28.0 (0.0, 50.0) | 3.0 (0.0, 53.0) | |

CRP: C-Reactive protein; DBP: Diastolic blood pressure, mBT shunt: modified Blalock-Taussig shunt, RV-PA shunt: Right ventricular pulmonary artery shunt, SBP: Systolic blood pressure, WBC: White blood cell count

*Kruskal–Wallis

†Chi Square

### Changes in overall health and cumulative hospitalization

No statistically significant differences were observed in any of the vital signs (weight, heart rate, or oxygen saturation) used to assess changes in overall health, either in the short or long term. Overall, patients experienced an increase in weight of 1.2 kg at three months and 3.5 kg at twelve months. Heart rate and oxygen saturation did not significantly change in any of the groups. The detailed results of these measurements are presented in Table 6. Additionally, the two groups had no significant difference in cumulative hospitalization. The median number of days in the hospital was 8.5 (IQR 6.0, 22.0) for the treatment group and 10.0 (IQR 6.0, 16.0) days for the control group, as shown in Table 6.

**Table 2** ANCOVA model for primary endpoints. Right ventricular cardiac function was adjusted for baseline values at three and twelve months

| Visits Compared | Cardiac Function | *n* | Treatment group LS Means Estimate (95% CI) | *n* | Control group LS Means Estimate (95% CI) | *P* value (adjusted*) |
|---|---|---|---|---|---|---|
| **Preop to 3 months** | Apical FAC | 40 | -2.225 (-3.898, -0.552) | 41 | -2.511 (-4.164, -0.895) | 0.80 (1.00) |
| | Circumferential Strain | 30 | 1.341 (-0.310, 2.992) | 35 | 0.170 (-1.358, 1.699) | 0.302 (1.00) |
| | Longitudinal Strain | 39 | 0.539 (-0.607, 1.686) | 39 | -1.240 (-2.387, -0.094) | **0.032** (0.19) |
| **Preop to 12 months** | Apical FAC | 33 | -3.897 (-5.680, -2.114) | 32 | -4.431 (-6.241, -2.620) | 0.676 (1.00) |
| | Circumferential Strain | 24 | 0.852 -1.001, 2.706) | 20 | 2.159 (0.128, 4.190) | 0.343 (1.00) |
| | Longitudinal Strain | 31 | 0.516 (-0.638, 1.715) | 32 | -0.453 (-1.633, 0.727) | 0.254 (1.00) |

ANCOVA: Analysis of covariance; FAC: Fractional area change

*Primary endpoints adjusted via the Bonferroni method to preserve the type I error rate

**Table 3** ANCOVA model for primary endpoints and troponins adjusted for baseline tricuspid regurgitation, stage I surgical palliation, and cardiopulmonary bypass time

| Visits Compared | Cardiac Function | *n* | Treatment group LS Means Estimate (95% CI) | *n* | Control group LS Means Estimate (95% CI) | *P* value |
|---|---|---|---|---|---|---|
| **Preop to 3 h postsurgery** | Troponin T | 36 | 0.790 (0.665, 0.915) | 36 | 0.595 (0.470, 0.720) | **0.037** |
| **Preop to 6 h postsurgery** | Troponin T | 39 | 0.700 (0.602, 0.798) | 35 | 0.539 (0.436, 0.643) | **0.032** |
| **Preop to 3 months** | Apical FAC | 39 | -2.401 (-4.144, -0.658) | 40 | -2.444 (-4.164, -0.723) | 0.972 |
| | Circumferential Strain | 30 | 1.216 (-0.489, 2.922) | 34 | 0.070 (-1.526, 1.667) | 0.342 |
| | Longitudinal Strain | 38 | 0.646 (-0.493, 1.786) | 38 | -1.555 (-2.695, -0.415) | **0.009** |
| | Troponin T | 13 | 0.417 (-1.154, 1.988) | 14 | 0.425 (-1.075, 1.926) | 0.994 |
| **Preop to 12 months** | Apical FAC | 33 | -3.792 (-5.589, -1.994) | 31 | -4.598 (-6.454, -2.743) | 0.536 |
| | Circumferential Strain | 24 | 0.957 (-1.037, 2.953) | 19 | 1.887 (-0.372, 4.147) | 0.548 |
| | Longitudinal Strain | 31 | 0.489 (-0.737, 1.715) | 31 | -0.495 (-1.722, 0.731) | 0.263 |
| | Troponin T | 13 | -0.024 (-0.025, -0.023) | 11 | -0.025 (-0.026, -0.024) | 0.147 |

ANCOVA: analysis of covariance; FAC: fractional area change

### ITQOL-97 and ASQ-3 assessment tools

Compared with the baseline ASQ-3 assessment tool, which revealed greater performance in the gross motor and personal social domains for the UCB-MNC group, no statistically significant differences were noted in these domains or any of the remaining domains assessed by the ASQ-3 and the 13 domains evaluated by the ITQOL-97 assessment tool at twelve months. (See Supplementary Tables S4 and S5, Additional file 1).

### Fatalities

At the one-year follow-up, five subjects had died, three in the treatment group and two in the control group; none prompted the activation of any adverse event-stopping rule, and none triggered mandatory reporting to the FDA. The causes of death involved multisystem failure, bradycardic arrest, and respiratory and heart failure. Notably, all these incidents occurred more than 60 days after surgery, ranging from five to twelve months. Notably, the site principal investigator deemed all deaths unrelated to the product or delivery procedure. The Data

**Table 4** Serious adverse events, comparison by group

| | Treatment group (*n* = 50) | Control group (*n* = 45) | *P* value |
|---|---|---|---|
| **Did the subject have at least one SAE within 1- month of enrollment?** | | | 0.0568* |
| Yes | 24 (48.0%) | 13 (28.9%) | |
| No | 26 (52.0%) | 32 (71.1%) | |
| **Did the subject have at least one SAE within 3- months of enrollment?** | | | **0.048*** |
| Yes | 29 (58.0%) | 17 (37.8%) | |
| No | 21 (42.0%) | 28 (62.2%) | |
| **Did the subject have at least one SAE within 12- months of enrollment?** | | | 0.143* |
| Yes | 34 (68.0%) | 24 (53.3%) | |
| No | 16 (32.0%) | 21 (46.7%) | |

SAE: Serious adverse event
*Chi-Square p value.

Safety and Monitoring Board did not recommend stopping or modifying the trial because of safety concerns.

## Discussion

This phase IIb clinical trial was designed as a follow-up study to support and broaden the findings of our previous phase I trial, which was published in 2019 and later expanded in 2021. This trial examined the safety and feasibility of intramyocardial injections of an autologous UCB-MNC product in HLHS patients [18, 19]. This initial study revealed no safety concerns over the six-month follow-up, along with preservation of right ventricular cardiac function and normalized growth rates in ten patients. In the present study, we further wanted to evaluate the efficacy of the autologous UCB-MNC product in a controlled trial. Our findings did not reveal significantly superior cardiac outcomes determined by detrimental cardiac strain in the shortterm following product administration. While safety endpoints were evaluated, our assessment was not exhaustive. Notably, our study uncovered no safety issues or concerns associated with the product's usage. Additionally, we did not observe any benefits regarding cumulative hospitalization, clinical outcomes, or parental perceptions of overall health or the ability of treated subjects to achieve better developmental skills.

We generated the first data-based efficacy clinical study concerning autologous intramyocardial injections of a UCB-MNC product in patients with HLHS. This study is also the largest reported clinical trial of stem cell therapy in HLHS patients.

Although it is an emerging field, significant advances and ongoing efforts have encouraged our urge to provide more robust evidence and strategies to help guide clinical decisions. In 2017, the PERSEUS clinical trial, a phase IIb study, included thirty-four subjects receiving intracoronary infusion of autologous cardiac progenitor cells [20]. They concluded that there was a significant and favorable improvement in cardiac function among the treated individuals, as observed through various imaging modalities at three and twelve months. This beneficial effect was also noticeable in late-treated subjects. Notably, at twelve months, all groups demonstrated an increase in the ejection fraction, as evidenced by increased global strain and strain rate and reduced ventricular volume. An additional study, the ELPIS phase I clinical trial, probed safety and suggested favorable right ventricular performance at one year of intramyocardial cell-based therapy using allogeneic bone marrow-derived mesenchymal stem cells (Lomecel-B) during stage II palliation surgery. As phase IIb trials continue to enroll patients, the medical community is encouraged by the potential of these findings.

In recent years, extensive evidence has supported stem cell therapies from perinatal sources, such as UCB and umbilical cord tissue (UCT). Products derived from these sources have shown a positive impact on the cardiovascular system, primarily through regenerative paracrine mechanisms [21–23]. Our study focused on developing a product derived from UCB-MNCs, combining various stem cell types to enhance therapeutic outcomes.

While UCB primarily contains hematopoietic cells, it also contains mesenchymal stem cells (MSCs), which is not a primary source. Historical evidence indicates that UCB is a challenging source of MSCs due to the low cell

**Table 5** Serious adverse events, comparison by groups among those with one or more SAEs

| | Treatment group (*n* = 50) | Control group (*n* = 45) | *P* value |
|---|---|---|---|
| **Number of SAEs within 1- month of enrollment (n)** | 24 | 13 | 0.17* |
| Median (Q1, Q3) | 2.0 (1.0, 6.0) | 1.0 (1.0, 4.0) | |
| **Number of SAEs within 3- months of enrollment (n)** | 29 | 17 | 0.18* |
| Median (Q1, Q3) | 2.0 (1.0, 8.0) | 1.0 (1.0, 7.0) | |
| **Number of SAEs within 12- months of enrollment (n)** | 34 | 24 | 0.16* |
| Median (Q1, Q3) | 2.0 (1.0, 13.0) | 1.5 (1.0, 9.0) | |

SAEs: Serious adverse events
*Wilcoxon rank sum p value.

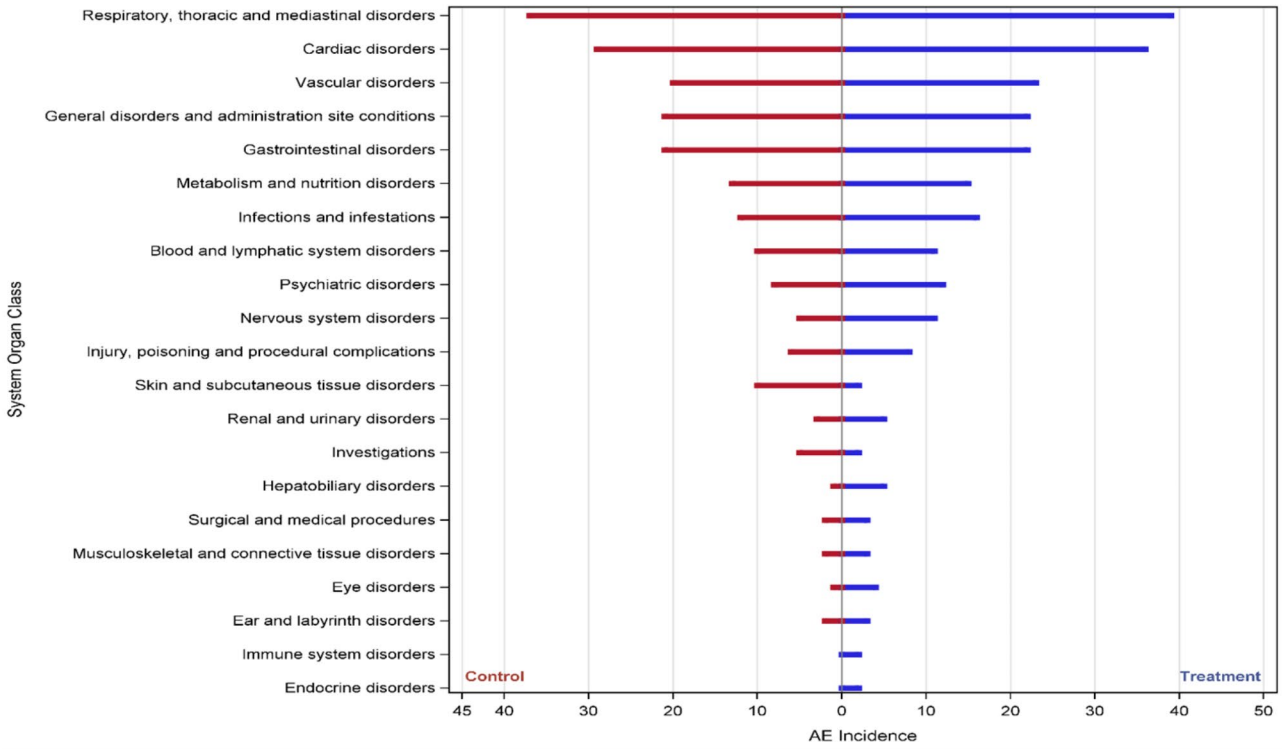


**Fig. 2** Adverse event incidence within one year of enrollment by system organ

**Table 6** Cumulative hospitalization at one and three months and changes in vital signs from baseline to three and twelve months after surgery

| | Treatment group (*n* = 50) | Control group (*n* = 45) | *P* value |
|---|---|---|---|
| **Change in vital signs (weight, heart rate, O2 saturation)** | | | |
| **Change in weight at 3 months (n)** | 44 | 39 | 0.956* |
| Median (Q1, Q3) | 1.2 (0.9, 1.5) | 1.2 (0.7, 1.8) | |
| **Change in weight at 12 months (n)** | 40 | 36 | 0.277* |
| Median (Q1, Q3) | 3.5 (2.9, 4.3) | 3.5 (2.3, 4.2) | |
| **Change in heart rate at 3 months (n)** | 43 | 38 | 0.909* |
| Median (Q1, Q3) | 5.0 (-8.0, 14.0) | 3.0 (-9.0, 17.0) | |
| **Change in heart rate at 12 months (n)** | 37 | 34 | 0.259* |
| Median (Q1, Q3) | -10.0 (-13.0, 8.0) | 1.0 (-12.0, 14.0) | |
| **Change in O2 saturation at 3 months (n)** | 40 | 36 | 0.639* |
| Median (Q1, Q3) | 4.0 (1.0, 8.5) | 5.5 (0.5, 9.5) | |
| **Change in O2 saturation at 12 months (n)** | 38 | 29 | 0.301* |
| Median (Q1, Q3) | 4.0 (0.0. 9.0) | 2.0 (-3.0, 8.0) | |
| **Cumulative Hospitalization** | | | |
| **At 1 month, days** | | | 0.776* |
| Median (Q1, Q3) | 8.5 (6.0, 22.0) | 10.0 (6.0, 16.0) | |
| **At 3 months, days** | | | 0.878* |
| Median (Q1, Q3) | 8.5 (6.0, 22.0) | 10.0 (6.0, 16.0) | |

*Kruskal–Wallis.

counts per cord blood unit and their limited proliferation rates compared to cord tissue [24–26]. In contrast, UCT is rich in MSCs, which can differentiate into various cell types, including adipocytes, osteoblasts, myocytes, and others. These limitations pose a significant barrier to utilizing human MSCs from UCB in regenerative medicine. Furthermore, MSCs derived from UCT differ significantly in functional properties and characterization compared to those derived from UCB. Products from UCT require additional processing steps such as extraction and cellular expansion to achieve therapeutic outcomes.

MSCs from UCT have shown the ability to produce exosomes, which is an acellular product that according to the evidence, is responsible for triggering molecular cascades involved in muscle repair, either cardiac or skeletal [27–29]. Both UCB-MNCs and MSCs from UCT share a promising potential for cardiovascular regeneration, with each therapy offering unique regenerative and anti-inflammatory benefits [30]. Together, they represent complementary approaches that aim to repair damaged cardiac tissues, enhance healing, and ultimately improve patient outcomes, paving the way for a future where regenerative medicine becomes a standard in cardiovascular care.

Our study has several notable strengths. Its multicenter design from multiple dispersed pediatric hospitals around the United States facilitated the generation of a substantial sample size, thereby enhancing the applicability and generalizability of the findings to real-world scenarios.

Our results did not provide evidence to demonstrate advantages in cardiac function and support our hypothesis explicitly. Instead, we observed that the administration of the autologous UCB-MNC product seemed to detrimentally affect right ventricular cardiac strain in the short term compared with the controls. Despite the shorter cardiopulmonary bypass (CPB) time in the treatment group, which could raise concerns regarding its association with the administration of the product, the analysis was adjusted for CPB time, among other covariates, to mitigate any confounding effects. As a result, the initially observed detrimental cardiac function persisted only in the short term. We hypothesize that this detrimental effect could be related to transient and localized needle injury during product administration, which is subclinical and not damaging enough to persist in time and increase the level of cardiac biomarkers. This apparent cardiac dysfunction was resolved by twelve-month echocardiography.

Furthermore, the UCB collection protocol of this study is aligned with that of our phase I clinical trial, ensuring a collection of over 35 mL of UCB that guarantees a success rate exceeding 90%. Despite previous assertions that this study represents the largest clinical trial on stem cell therapy for HLHS, there might be an essential sample size factor limiting the outcomes. In 2020, the FUEL trial in a cohort of 400 subjects with single-ventricle CHD who had undergone Fontan surgery and were treated with either udenafil or placebo at 26 weeks did not detect a difference between groups when the myocardial performance index was assessed [31]. These findings might suggest that, despite a large sample size, additional measures might be necessary to identify subtle differences. We recommend an extended follow-up, which could yield long-term benefits beyond the current observations and contribute encouraging evidence to the literature on stem cell therapy.

Additionally, we observed a decrease of up to 6% in the apical FAC in the control group at twelve months, which is consistent with our previous findings using natural history data, which reported a 5% decrease in FAC six months after stage II palliation surgery in controls [19]. While cMRI remains the gold standard for evaluating right ventricular cardiac function in CHD patients because of its superior spatial visualization of anatomical structures, studies support the complementary roles of 2-D echocardiography and cMRI, each contributing to limitations [32, 33]. Our data support the natural history of right ventricular cardiac function via echocardiography and suggest more assertive imaging methods to further evaluate FAC and other measures of RV cardiac function, volume, and size.

### Limitations

First, the primary analysis relied on the standard of care echocardiography as the imaging assessment tool. While this method is dependable, it may provide less precise and accurate right ventricular cardiac function measurements than a more thorough and reproducible tool such as cardiac magnetic resonance imaging (cMRI). Although cMRI was specified, we amended our protocol during the COVID-19 pandemic to make cMRI optional at follow-up to comply with restrictions on elective procedures at most clinical centers. We do not have sufficient cMRI to perform a meaningful statistical assessment. Second, the study design focused exclusively on analyzing a homogenous cohort of patients with preserved baseline cardiac function, excluding those with abnormal baseline right ventricular cardiac function, which would have allowed for a more sensitive assessment of the potential benefit of the UCB-MNC product. Third, this study was nonrandomized to best utilize valuable UCB-MNC products, introducing inherent biases that we tried to minimize through rigorous patient selection and a blinded assessment of imaging outcomes, ensuring the reliability of our findings.

Our continuous efforts provide fertile ground for further investigation, encouraging researchers to identify

areas for improvement, assess strengths and weaknesses, and explore potential strategies. This endeavor aims to facilitate reevaluating existing approaches and developing novel hypotheses, shedding light on the practical applicability of UCB-MNC products.

## Conclusions

In conclusion, this phase IIb clinical trial of intramyocardial injections of autologous UCB-MNC products did not reveal superior cardiac outcomes in HLHS patients during stage II palliation surgery. There was an unfavorable outcome regarding cardiac function, as the product failed to improve right ventricular cardiac strain in the short and long term compared with controls. The safety profile of the UCB-MNC product remains acceptable, and no serious safety issues or concerns have been identified. Further randomized, placebo-controlled, and blinded studies addressing our limitations are needed. Our team is committed to continuing to study the effectiveness of novel stem cell therapies, specifically UCB-MNC, in children with CHD. We are undertaking novel initiatives to overcome study limitations and facilitate a more precise and comprehensive evaluation of the UCB-MNC product's long-term benefits. Additionally, we aimed to assess other critical long-term outcomes, such as completing stage III palliation and the time to heart transplantation.

### Abbreviations

| | |
|---|---|
| ANCOVA | Analysis of covariance |
| ASQ-3 | Age and Stage Questionnaire-3 |
| cMRI | Cardiac magnetic resonance imaging |
| DMSO | Dimethyl sulfoxide |
| eCRF | Electronic Case Report Form |
| FAC | Fractional area change |
| HLHS | Hypoplastic left heart syndrome |
| IRB | Institutional Review Board |
| ITQOL-97 | Infant Toddler Quality of Life Questionnaire-97 |
| mBT shunt | Modified Blalock-Taussig shunt |
| RV-PA shunt | Right ventricular pulmonary artery shunt |
| SAEs | Severe adverse events |
| UCB-MNC | Umbilical Cord Blood-derived Mononuclear Cells |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04316-3.

Supplementary Material 1

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### Author contributions

Design of the study (TJN, SCP, CH), data collection (HLHS consortium), statistical analysis (DWS), writing-original draft and elaboration of tables (CGN, CH, DWS), writing-review & editing (TJN, CH), critical review (HMB, WFC, DLC, MYQ, JWR). All authors read and approved the last version of the manuscript and agreed to be responsible for all aspects of the clinical trial.

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### Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. All materials used in the study are available upon request.

## Declarations

### Ethics approval and consent to participate

The study titled "Phase IIb Study of Intramyocardial Injection of Autologous Umbilical Cord Blood-Derived Mononuclear Cells during Stage II Surgical Repair of Hypoplastic Left Heart Syndrome (Auto Cell-II)" was individually approved by the Institutional Review Boards (IRBs) at each participating site under protocol number CSP-4401 and IND 15343 on August 30, 2018.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Artificial intelligence

The authors declare that they have not used AI-generated work in this manuscript.

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