AUTOLOGOUS UMBILICAL CORD BLOOD STEM CELL THERAPY FOR HYPOPLASTIC LEFT HEART SYNDROME: A NON-RANDOMIZED CONTROL TRIAL OF THE EFFICACY AND SAFETY OF INTRAMYOCARDIAL INJECTIONS

PHASE IIB CLINICAL TRIAL

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BACKGROUND

- HLHS treatment: palliative multistage surgical repair
- Despite advancements and improved outcomes compared to previous decades, patients are at risk of long-term morbidity and mortality with cardiac dysfunction, and eventual heart transplantation
- Regenerative therapy as adjuvant therapy to minimize the risks associated with ventricular failure
- Stem cell therapy goal: to enhance the function of the native ventricle of these children

Our team efforts:

- Phase I clinical trial: 10 subjects were successfully treated with UCB-MNC product and followed up over two years. Favorable outcomes: one single adverse event related to cell delivery (an injection site epicardial bleed that required simple oversewn. No other significant safety concerns.
- Preservation of RV function (at 6 months) and normalized growth rates[1]

[1] Burkhart HM, Qureshi MY, Rossano JW, Cantero Peral S, O'Leary PW, Hathcock M, Kremers W, Nelson TJ; Wanek HLHS Consortium Clinical Pipeline. Autologous stem cell therapy for hypoplastic left heart syndrome: Safety and feasibility of intraoperative intramyocardial injections. J Thorac Cardiovasc Surg. 2019 Dec;158(6):1614-1623. doi: 10.1016/j.jtcvs.2019.06.001. Epub 2019 Jun 7. PMID: 31345560.

OBJECTIVE

- To determine the efficacy of autologous intramyocardial injections of an Umbilical Cord Blood-derived Mononuclear cells (UCB-MNC) product, at the time of *stage II palliation surgery* in subjects with hypoplastic left hearts.

METHODS

- Multicenter (HLHS consortium), prospective, open-label, non-randomized study.
- 50 treated subjects and 45 controls (same selection criteria except for UCB-MNC acceptability).
- UCB collected at birth. Processed by ReGen Theranostics Inc., to manufacture UCB-MNC product
- Inclusion
    - Diagnosis of HLHS and history of stage I surgical repair (Norwood procedure)
    - Scheduled for stage II palliation surgery at less than 13 months of age
    - UCB-MNC product considered acceptable for clinical use (treatment arm)
- Exclusion criteria
    - History of dimethyl sulfoxide reaction (DMSO) sensitivity (treatment arm).
    - Parent(s) and/or legal guardian(s) unwilling to have their child participate.
    - Severe chronic diseases, extensive extra-cardiac syndromic features, known history of cancer, and other complications related to the HLHS diagnosis.

METHODS

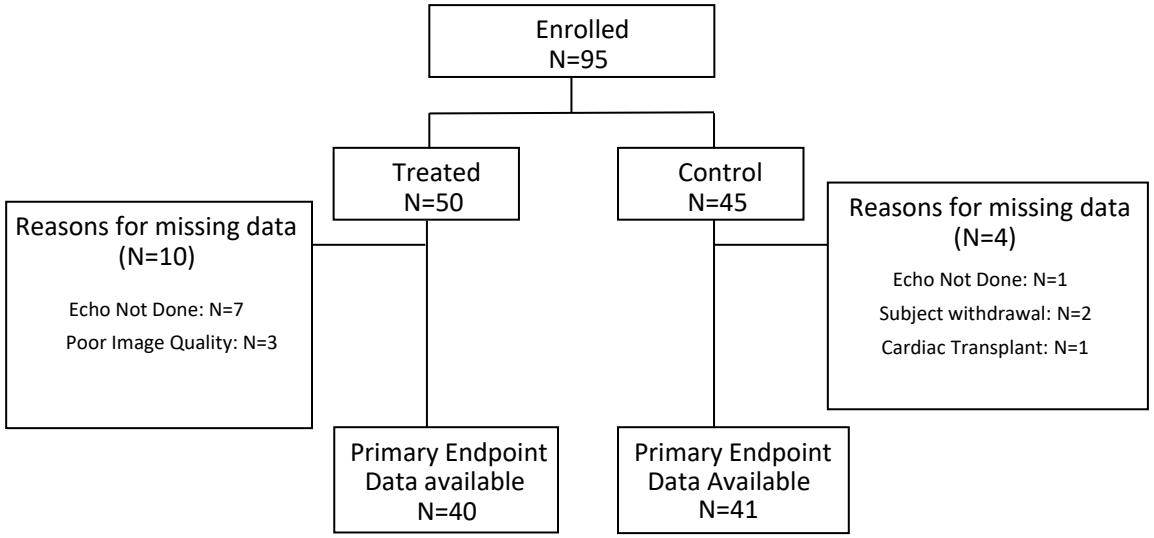
STUDY PRODUCT, DOSE, ROUTE, TIMING

- Product: Autologous UCB-MNC derived from umbilical cord blood at a concentration of 10-30 million TNC/mL
- Dose: 0.1 mL/kg body weight -> 1-3 million TNC/kg body weight
- Route: Subepicardial injections in the right ventricle
- Time: Single treatment administered intraoperatively upon completion of stage II surgery (Glenn procedure)

DATA ACQUISITION

- Demographic and operative data
- Baseline and postoperative clinical and laboratory data
  - Physical examination, vital signs, telemetry monitoring, blood workup including CBC, LFTs, renal function, TSH, and cardiac biomarkers
- Baseline and postoperative cardiac function with transthoracic echocardiography (at 3 and 12 months)
  - We used a blinded imaging core lab (Mayo Clinic staff not affiliated with this clinical trial) to evaluate the echocardiography images.

CONSORT FLOW DIAGRAM



Consort Flow Diagram flow of the phase IIB clinical trial showing the number of enrolled subjects., subjects' allocation into treatment and control groups. Primary Endpoint data available after ruling out missing data.

ENDPOINTS

Primary endpoints

- Short-term (3-months) changes in right ventricular cardiac function
  - Apical Fractional Area Change (FAC)
  - Circumferential strain
  - Longitudinal strain
- Long-term (12-months) changes in right ventricular cardiac function

Secondary endpoints

- Change in overall health (vital signs: weight, heart rate, and SO2) at 3 and 12 months
- Cumulative incidence of hospitalization at 1 and 3 months
- Safety: severe adverse events (SAEs) at 1, 3, and 12 months

RESULTS

Demographics

	Treatment arm (n = 50)	Control arm (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			0.2734
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)	
SPO2 (%)			0.590
Median (Q1, Q3)	80.5 (77, 84)	80.0 (78, 84)	

Baseline cardiac function and cardiac biomarkers

	Treatment arm (n = 50)	Control arm (n = 45)	P-value
Cardiac function as assessed by echocardiography (TTE)			
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 Markers			
Troponin-T, ng/mL (n)	42	41	0.376
Median (Q1, Q3)	0.025 (0.018, 0.039)	0.024 (0.013, 0.038)	
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)	
C-reactive protein, mg/L (n)	49	42	0.105
Median (Q1, Q3)	5.0 (2.9, 5.0)	5.0 (2.5, 6.0)	

Baseline blood workup

- No statistically significant difference
- CBC w/differential: WBC count, Hb, Hct, platelets
- Liver/Renal function: ALT, AST, ALKP, proteins, BUN, Cr
- Thyroid function: TSH

Type of surgery and operative variables:

	Treatment arm (n = 50)	Control arm (n = 45)	P-value
Stage I surgery type			**0.049**
Norwood procedure with mBT shunt	8 (16.0%)	5 (11.1%)	
Norwood procedure with non-valved 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)	

RESULTS: ENDPOINTS

- **Primary endpoints:** Short- and long-term changes in right ventricular cardiac function

Visits Compared	Cardiac Function	n	Treatment LS Means Estimate (95% CI)	n	Control LS Means Estimate (95% CI)	P-value
Pre-op to 3h post-surgery	Troponin T	38	0.747 (0.623, 0.870)	37	0.641 (0.516, 0.766)	0.236
Pre-op to 6h post-surgery	Troponin T	41	0.665 (0.559, 0.772)	36	0.582 (0.468, 0.696)	0.293
Pre-op to 3 mo	Apical FAC	40	-2.225 (-3.898, -0.552)	41	-2.511 (-4.164, -0.895)	0.809
	Circumferential Strain	30	1.341 (-0.310, 2.992)	35	0.170 (-1.358, 1.699)	0.302
	Longitudinal Strain	39	0.539 (-0.607, 1.686)	39	-1.240 (-2.387, -0.094)	**0.032**
	Troponin T	13	-0.088 (-1.437, 1.271)	14	0.889 (-0.414, 2.193)	0.301
Pre-op to 12 mo	Apical FAC	33	-3.897 (-5.680, -2.114)	32	-4.431 (-6.241, -2.620)	0.676
	Circumferential Strain	24	0.852 (-1.001, 2.706)	20	2.159 (0.128, 4.190)	0.343
	Longitudinal Strain	31	0.516 (-0.638, 1.715)	32	-0.453 (-1.633, 0.727)	0.254
	Troponin T	13	-0.023 (-0.024, -0.022)	12	-0.024 (-0.025, -0.023)	0.091

ANCOVA model adjusted for its baseline values.

RESULTS: ENDPOINTS

- **Primary endpoints:** Short- and long-term changes in right ventricular cardiac function

Visits Compared	Cardiac Function	n	Treatment LS Means Estimate (95% CI)	n	Control LS Means Estimate (95% CI)	P-value
Pre-op to 3h post-surgery	Troponin T	36	0.79 (0.66, 0.91)	36	0.59 (0.47, 0.72)	**0.037**
Pre-op to 6h post-surgery	Troponin T	39	0.70 (0.60, 0.79)	35	0.53 (0.43, 0.64)	**0.032**
Pre-op to 3mo	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	0.41 (-1.15, 1.98)	14	0.42 (-1.07, 1.92)	0.994
Pre-op to 12mo	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	-0.02422 (-0.025, -0.023)	11	-0.02547 (-0.026, -0.024)	0.147

ANCOVA model adjusted for its baseline values and for baseline tricuspid regurgitation, stage I surgical palliation, and cardiopulmonary bypass time.

	Treatment arm (n = 50)	Control arm (n = 45)	Total (n = 95)	P-value
Baseline BNP, pg/mL (n)	48	43	91	0.450
Median (Q1, Q3)	1296.0 (687.5, 3066.0)	1200.0 (855.0, 2196.0)	1277.0 (808.0, 2590.0)	
3-month BNP, pg/mL (n)	23	17	40	0.837
Median (Q1, Q3)	977.0 (438.0, 2078.0)	850.0 (486.0, 1404.0)	946.5 (469.0, 1681.0)	
12-month BNP, pg/mL (n)	47	40	87	0.539
Median (Q1, Q3)	557.0 (312.0, 1090.0)	375.0 (251.0, 811.0)	498.5 (264.0, 811.0)	

RESULTS: ENDPOINTS

- **Secondary endpoints:** Change in overall health (vital signs: weight, heart rate, and SO2) in the short and long term, and cumulative incidence of hospitalization at 1 and 3 months.

	Treatment arm (n = 50)	Control arm (n = 45)	Total (n = 95)	P-value
Change in weight,				0.956
3 months (n)	44	39	83	
Median (Q1, Q3)	1.2 (0.9, 1.5)	1.2 (0.7, 1.8)	1.2 (0.8, 1.6)	
Change in weight,				0.277
12 months (n)	40	36	76	
Median (Q1, Q3)	3.5 (2.9, 4.3)	3.5 (2.3, 4.2)	3.5 (2.8, 4.2)	
Change in heart rate,				0.909
3 months (n)	43	38	81	
Median (Q1, Q3)	5.0 (-8.0, 14.0)	3.0 (-9.0, 17.0)	4.0 (-9.0, 15.0)	
Change in heart rate,				0.259
12 months (n)	37	34	71	
Median (Q1, Q3)	-10.0 (-13.0, 8.0)	1.0 (-12.0, 14.0)	-2.0 (-13.0, 12.0)	
Change in O2 saturation,				0.639
3 months (n)	40	36	76	
Median (Q1, Q3)	4.0 (1.0, 8.5)	5.5 (0.5, 9.5)	4.0 (0.5, 9.0)	
Change in O2 saturation,				0.301
12 months (n)	38	29	67	
Median (Q1, Q3)	4.0 (0.0. 9.0)	2.0 (-3.0, 8.0)	3.0 (-1.0, 8.0)	

	Treatment arm (n = 50)	Control arm (n = 45)	Total (n = 95)	P-value
Length of hospital stay at 1-month (days) Median (Q1, Q3)	8.5 (6.0, 22.0)	10.0 (6.0, 16.0)	9.0 (6.0, 18.0)	0.776
Length of hospital stay at 3-months (days) Median (Q1, Q3)	8.5 (6.0, 22.0)	10.0 (6.0, 16.0)	9.0 (6.0, 18.0)	0.878

SEVERE ADVERSE EVENTS (SAE)

	Treatment, (n = 50)	Control, (n = 45)	Total, (n = 95)	P-value
Did the subject have at least one SAE within 1- month of enrollment?				0.056
Yes	24 (48.0%)	13 (28.9%)	37 (38.9%)	
No	26 (52.0%)	32 (71.1%)	58 (61.1%)	
Did the subject have at least one SAE within 3- months of enrollment?				**0.048**
Yes	29 (58.0%)	17 (37.8%)	46 (48.4%)	
No	21 (42.0%)	28 (62.2%)	49 (51.6%)	
Did the subject have at least one SAE within 12- months of enrollment?				0.143
Yes	34 (68.0%)	24 (53.3%)	58 (61.1%)	
No	16 (32.0%)	21 (46.7%)	37 (38.9%)	

Number of severe adverse events (SAEs)	Treatment, (n = 50)	Control, (n = 45)	Total, (n = 95)	P-value
# SAEs within 1- month of enrollment (n)	24	13	37	0.178
Median (Q1, Q3)	2.0 (1.0, 6.0)	1.0 (1.0, 4.0)	1.0 (1.0, 6.0)	
# SAEs within 3- months of enrollment (n)	29	17	46	0.189
Median (Q1, Q3)	2.0 (1.0, 8.0)	1.0 (1.0, 7.0)	2.0 (1.0, 8.0)	
# SAEs within 12- months of enrollment (n)	34	24	58	0.166
Median (Q1, Q3)	2.0 (1.0, 13.0)	1.5 (1.0, 9.0)	2.0 (1.0, 13.0)	

6 deaths (4 treatment, 2 control arm)

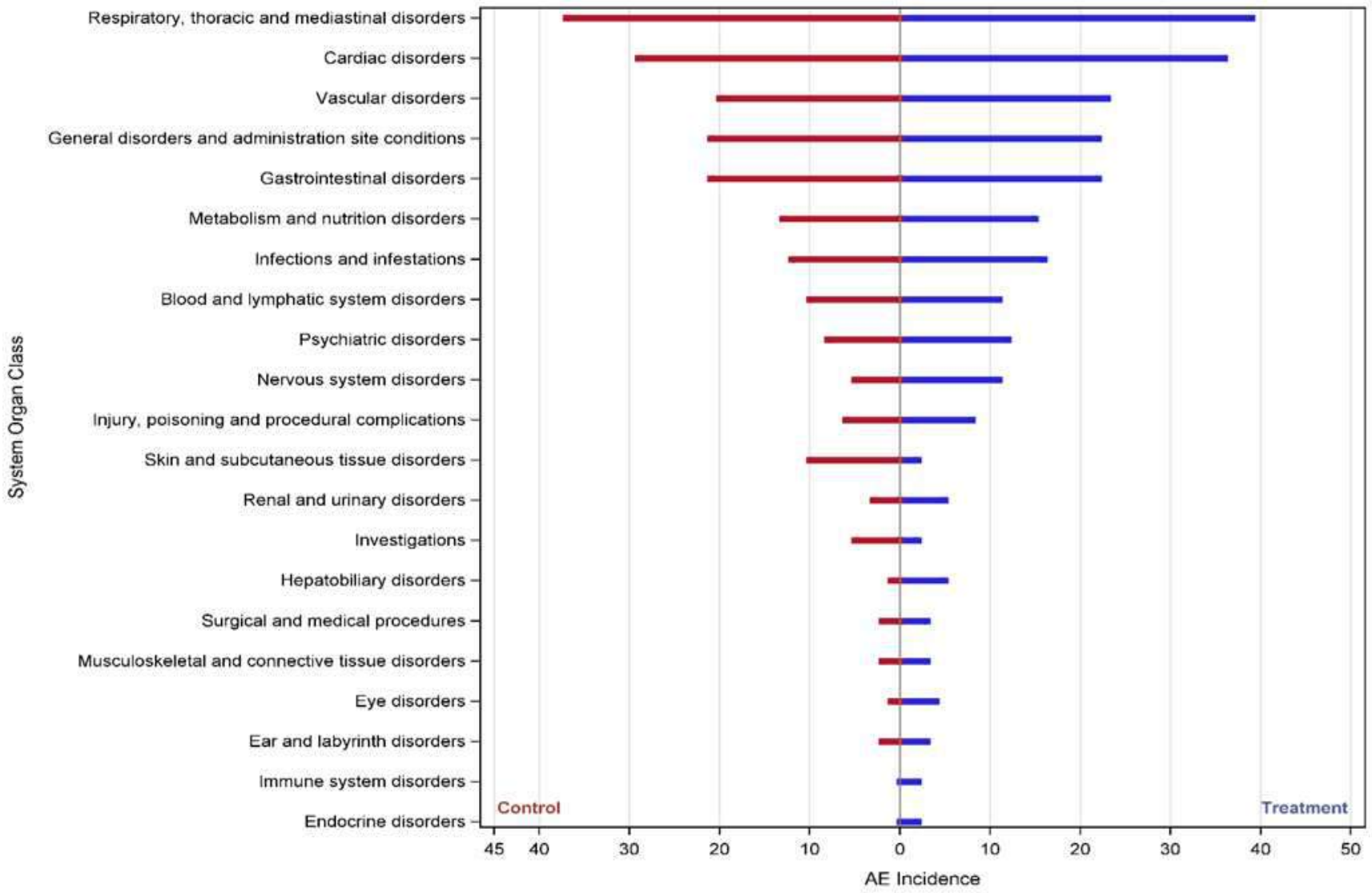
Not related to either the product or delivery procedure.

None triggered an AE stopping rule

> 60d post S-II surgery (5-12mo)

Causes: Multisystem failure, bradycardic arrest, respiratory and heart failure

US Department of Health & Human Services. Common Terminology Criteria for Adverse Events (CTCAE). Common Terminology Criteria for Adverse Events (CTCAE) (nih.gov)



Adverse events incident within 1 year of enrollment by system organ in each group

CONCLUSIONS

- Our UCB-MNC product did not demonstrate superior cardiac outcomes. No improvement in cardiac strain in the short and long-term.
- Further studies addressing our limitations are needed. (Randomized, placebo, and blinded).
- Our team is committed to further investigating the effectiveness of stem cell therapies, specifically UCB-MNC in children with congenital heart disease.
- We are undertaking novel initiatives to overcome our limitations and facilitate a more precise and comprehensive evaluation of our product’s long-term outcomes such as completing stage III palliation and time to heart transplantation.

THANK YOU

QUESTIONS & ANSWERS