Regenerative therapies from cord blood and associated birthing tissues

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Cell & Regenerative Therapies

- **Diseases:**
  - Alzheimer's Disease
  - Amyotrophic lateral sclerosis (ALS)
  - ARDS (COVID related)
  - Asthma
  - Autism
  - Bronchopulmonary dysplasia (BPD)
  - Cancer
  - Chronic Bronchiectasis
  - COVID MIS-C
  - CP
  - Crohns Disease
  - Diabetes
  - GvHD
  - HIE
  - Leukodystrophies
  - Limb ischemia
  - Multiple Sclerosis
  - Myocardial infarction
  - Osteoarthritis
  - Osteogenesis Imperfecta
  - Osteoporosis
  - Perianal fistulas
  - Pulmonary fibrosis
  - Spinal cord injury
  - Traumatic brain injury
  - Wound healing

- **Cell types**
  - BM MSC*
  - Adipose MSC
  - Cord Blood
    - HSCT
    - Immunotherapies
    - Neurologic repair
  - CT MSC
  - Exosomes
  - Placental MSC
  - Amniotic fluid MSCs
  - Amniotic membranes
- **Routes of Administration**
  - Intravenous
  - Intrathecal
  - Intracranial
  - Intra-articular (joint)
  - Intranasal
  - Intratracheal
  - Local injection

* Mesoblast BLA submitted for treatment of acute steroid refractory GVHD in children

Overview of ongoing work at Duke

We manufacture 3 types of therapeutic cells under GMP:

- Cord Blood
  - ✓Babies blood in placenta –auto, allo, related, unrelated sources
  - ✓FDA licensed public bank
  - ✓Monocytes are the active cell (MNCs)
  - ✓Used for HSCT and brain injury (CP, HIE, stroke)
- Cord Tissue MSCs
  - ✓Umbilical cord cells manufactured over 3 months
  - ✓Modulate and suppress inflammation
  - ✓Used in GVHD, ASD, COVID-ARDS & MIS-C, HIE, OA
- DUOC
  - ✓Cells manufactured from cord blood in 21 days
  - ✓Remyelinate the brain and other nerves
  - ✓Used in Leukodystrophies, MS (pending)

Key observations to date

CEREBRALPALSY + HYPOXIC ISCHEMIC ENCEPHALOPATHY

Active cells are cord blood monocytes

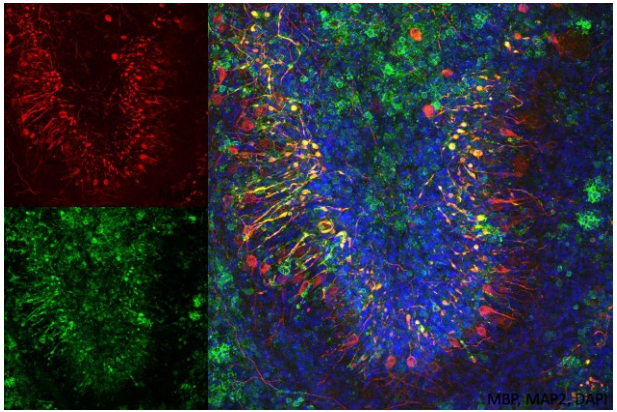
PLOS ONE

RESEARCH ARTICLE

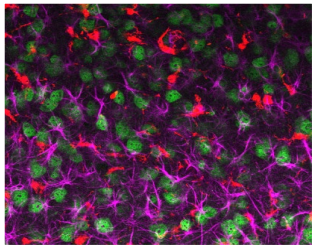
Human umbilical cord blood monocytes, but not adult blood monocytes, rescue brain cells from hypoxic-ischemic injury: Mechanistic and therapeutic implications

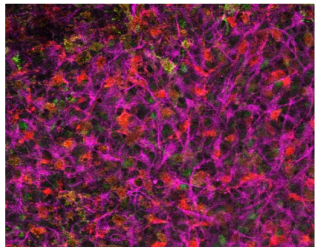
Arjun Saha*, Sachit Patel, Li Xu, Paula Scotland, Jonathan Schwartzman, Anthony J. Filiano, Joanne Kurtzberg, Andrew E. Balber

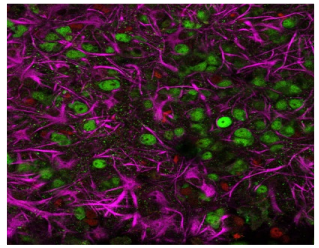
Marcus Center for Cellular Cures (MC3), Duke University School of Medicine, Durham, North Carolina, United States of America

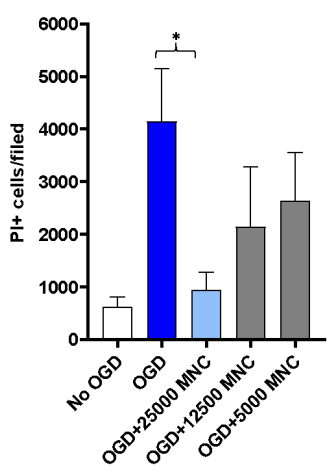


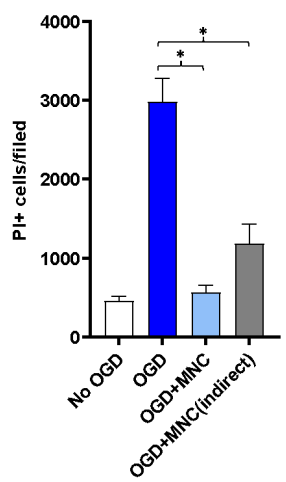
MAP2 MBP DAPI











Cerebral Palsy - Auto

Established dose of 25M/kg

STEM CELLS TRANSLATIONAL MEDICINE

CORD BLOOD

Authored by a member of CORD BLOOD ASSOCIATION

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Effect of Autologous Cord Blood Infusion on Motor Function and Brain Connectivity in Young Children with Cerebral Palsy: A Randomized, Placebo-Controlled Trial

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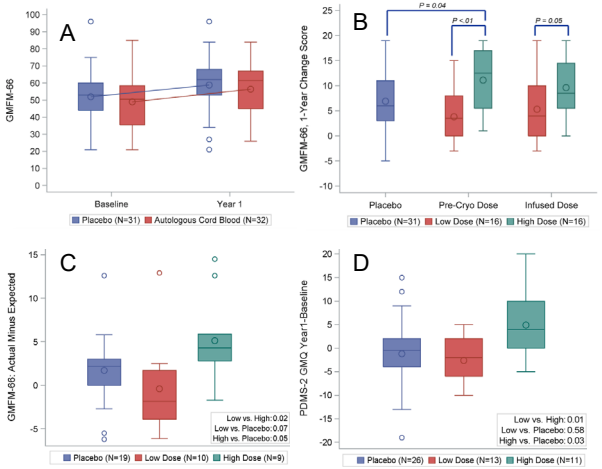
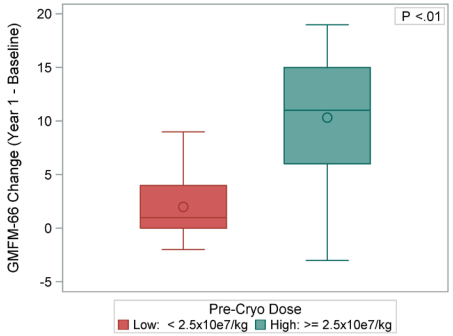
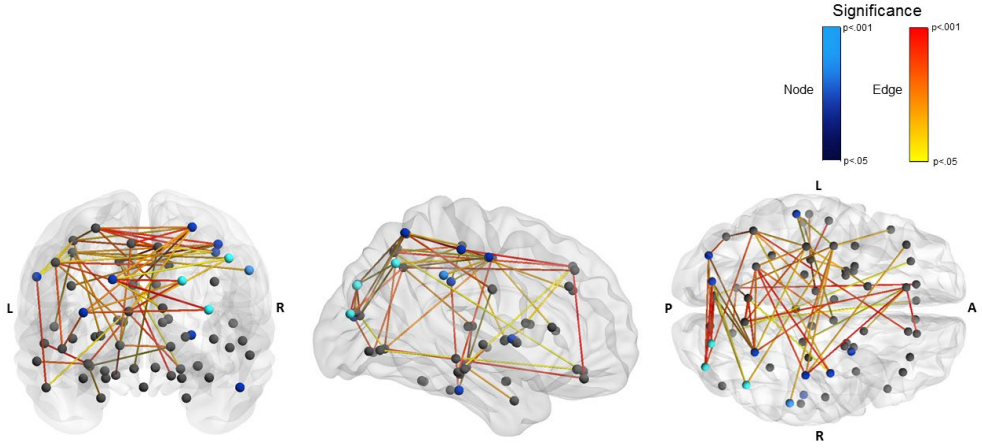
Key Words. Autologous stem cell transplantation • Cellular therapy • Clinical Trials • Cord blood • Human cord blood • Nervous system • Umbilical cord blood

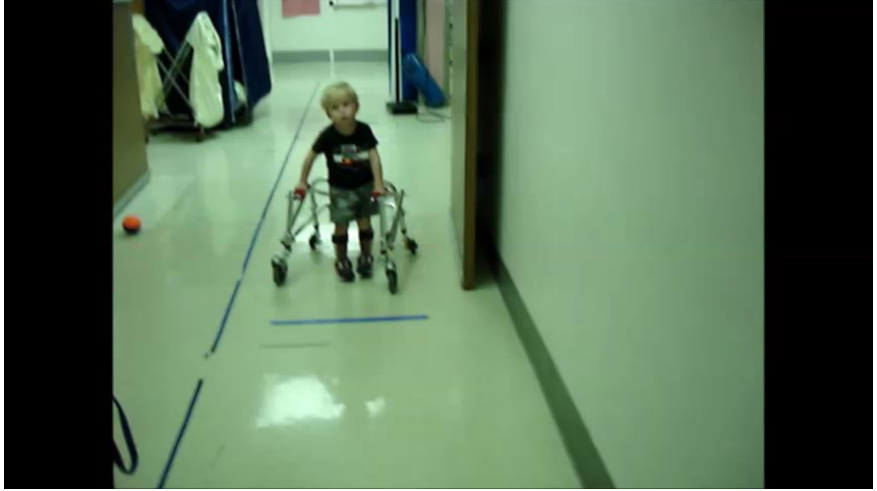




Siblings (haplo/full match)

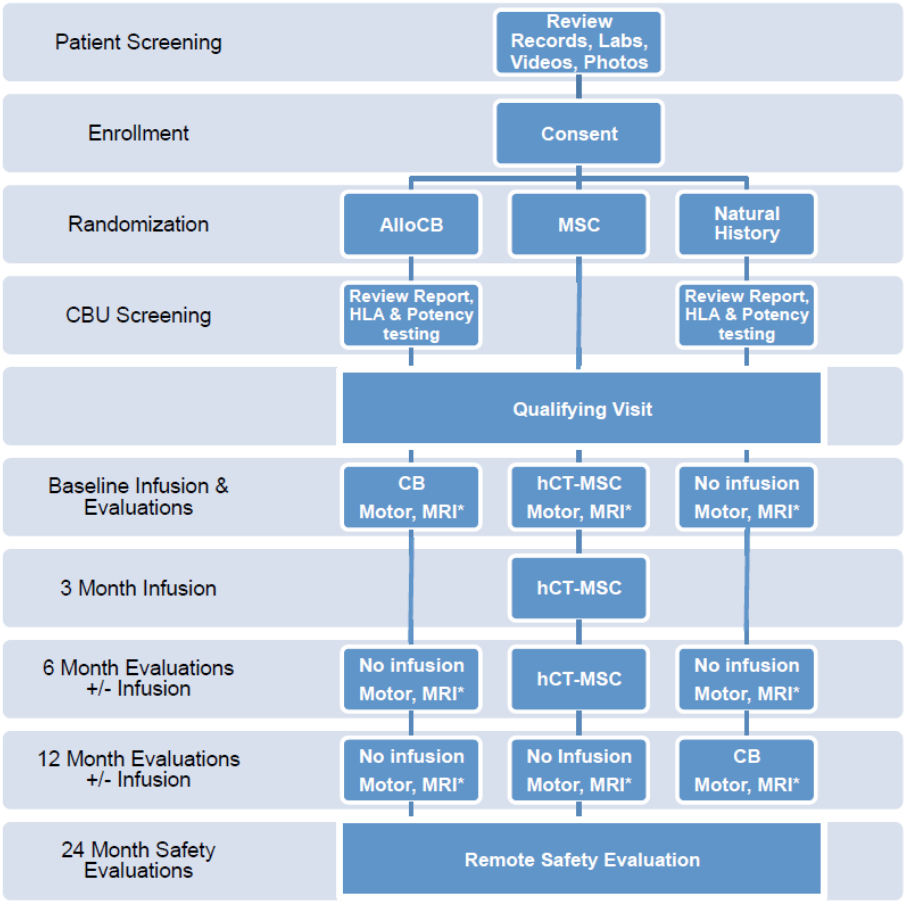
Measure	Baseline	Mean (SD) 6 months	Change score
GMFM-66	37.5 (10.1)	40.8 (8.8)	4.8 (2.5)#
PDMS – Gross Motor Quotient	47.7 (7.7)	48.7 (8.4)	1.0 (2.9)
PDMS – Fine Motor Quotient	63.3 (15.9)	63.4 (12.9)	0.1 (7.2)
AHA Interval Score	44.6 (20.4)	49.9 (19.6)	5.3 (3.2)





Increases in motor function at 1 year that were 30% higher than predicted for age and level of function were scored a response to cord blood cells.

ACCeNT-CP Allo CB (100) and hCT-MSC Phase 2 (N=90)



- 90 pediatric patients with CP
- Ages 2-5 years
- GMFCS levels of II-IV
- Randomized to high dose allogeneic unrelated cord blood, human cord tissue MSCs or natural history (control)
- Baseline, 6 and 12 month evaluations

ACCENT CP: GMFM-66 RESULTS

Cord blood, but not MSCs improved function in children with CP

	Six Months (N=90)						12 Months (N=68)				
	DF	Estimate	Standard Error	95% Confidence Limits		P-value	Estimate	Standard Error	95% Confidence Limits		P-value
AlloCB	1	1.37	0.96	-0.50	3.25	0.151	**3.26**	**1.36**	**0.59**	**5.93**	**0.017**
hCT-MSC	1	0.22	0.97	-1.68	2.13	0.818	1.45	1.31	-1.12	4.01	0.270

BabyBac 1: Survival with 1 yr Bayley III scores ≥ 85 in 3 domains

	Cells N = 28 N (%)	Cooled only N = 66 N (%)	p
Survival with all 3 Bayley domain scores ≥ 85	18 (64)	25 (38)	**0.04**
Bayley < 85 at one year (among survivors)*	9 (35)	23 (48%)	0.33

THE JOURNAL OF PEDIATRICS • www.jpeds.com ORIGINAL ARTICLES

Feasibility of Autologous Cord Blood Cells for Infants with Hypoxic-Ischemic Encephalopathy

C. Michael Cotten, MD[1], Amy P. Murtha, MD[2], Ronald N. Goldberg, MD[1], Chad A. Grotegut, MD[2], P. Brian Smith, MD[1], Ricki F. Goldstein, MD[1], Kimberley A. Fisher, PhD[1], Kathryn E. Gustafson, PhD[3], Barbara Waters-Pick, BS, MT(ASCP)[4], Geeta K. Swamy, MD[2], Benjamin Rattray, MD[1], Siddhartha Tan, MD[5], and Joanne Kurtzberg, MD[6]

J Pediatr 2014;164:973-9

BabyBAC 2: Randomized placebo controlled blinded study of auto CB infusion in babies with HIE

- Accrual difficulties, enrollment stopped at 37 babies
- *162 babies screened and 56% eligible - but didn't have cord blood collected*
  - *Difficult deliveries, abruption, etc*
- *Analysis of first 29 babies showed benefit for survival with normal function at 12 months. 3 vs 9 deaths in treated vs placebo groups (P=0.06)*
- *What about an 'off the shelf' allogeneic product for babies where CB is not collected?*

Phase 1 Trial of Allo-MSC's for babies with moderate to severe HIE

- **Subjects:**
  - > 35 weeks gestation with moderate to severe HIE
- **Intervention**
  - *Allogeneic umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC)*
  - Dosing
    - First 3 infants get 1 dose (cohort 1): 2 x 10^6 cells/kg i.v. in the first 48 postnatal hours
    - Second 3 infants get 1st dose plus 2nd dose at 2 months
- **Outcomes**
  - Safety: infusion reactions, infection, PRA
  - Assessing survival and neuro outcomes at 12 – 16 months
  - 6 babies enrolled and treated, 2 severe, 4 moderate – no safety issues
  - Hospital discharge 9-10 days; 9 day MRIs normal
  - 1 year follow up: all babies doing well
- **Next steps**
  - Phase 3, placebo controlled blinded trial, biological assignment to CB or MSC

Autism spectrum disorder Cord Blood

Key observations to date

STEM CELLS TRANSLATIONAL MEDICINE

CORD BLOOD

Autologous Cord Blood Infusions Are Safe and Feasible in Young Children with Autism Spectrum Disorder: Results of a Single-Center Phase I Open-Label Trial

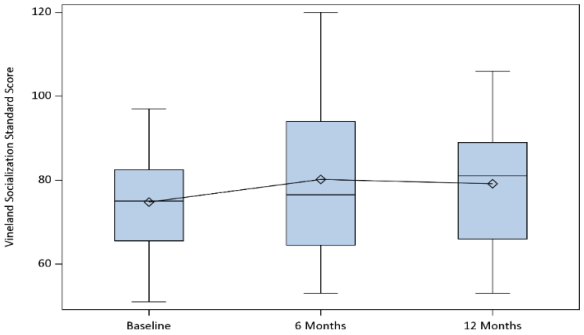
GERALDINE DAWSON,[a] JESSICA M. SUN,[b] KATHERINE S. DAVLANTIS,[a] MICHAEL MURIAS,[a,c] LAUREN FRANZ,[a] JESSE TROY,[b] RYAN SIMMONS,[b] MAURA SABATOS-DEVITO,[a] REBECCA DURHAM,[b] JOANNE KURTZBERG[b]

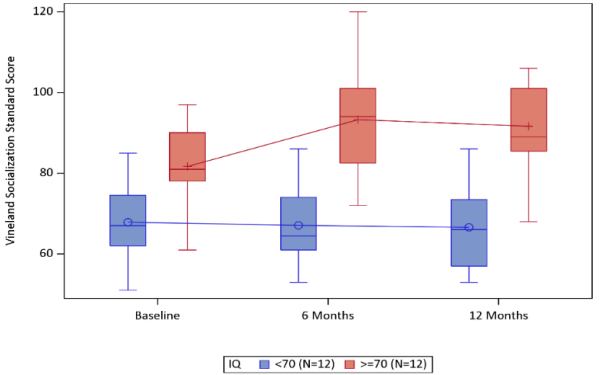
Authored by a member of CORD BLOOD ASSOCIATION

Key Words. Autism spectrum disorder • Autologous umbilical cord blood • Cell therapy

25 children with ASD
Ages 2-6, 80% males
Non-verbal IQ 35-123 (median 64)
Assess endpoints at 6 and 12 months
Assess feasibility and safety
Excluded children with genetic causes of autism
Tested endpoints for future phase 2 /3 trials

Primary endpoint: Vineland Adaptive Behavior Scale – Socialization Standard Score



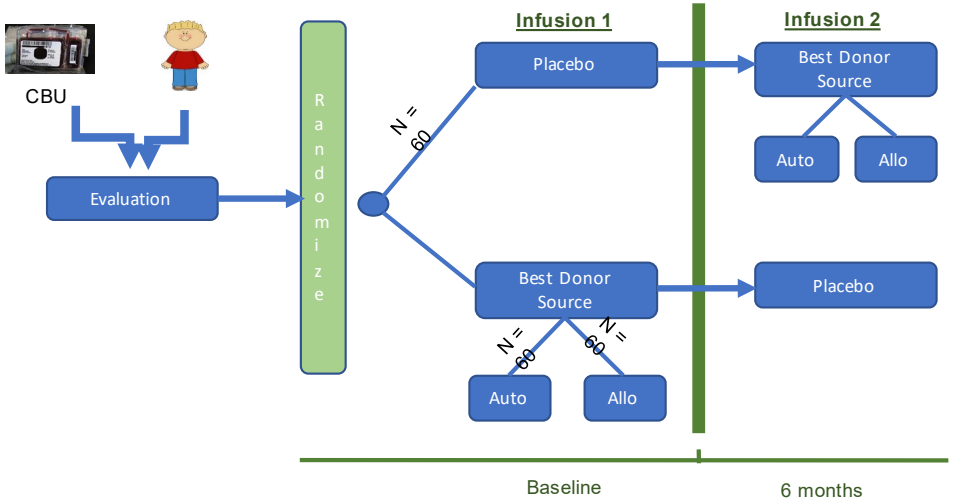


ARTICLE IN PRESS
THE JOURNAL OF PEDIATRICS • www.jpeds.com
ORIGINAL ARTICLES

A Phase II Randomized Clinical Trial of the Safety and Efficacy of Intravenous Umbilical Cord Blood Infusion for Treatment of Children with Autism Spectrum Disorder

Geraldine Dawson, PhD[1,2], Jessica M. Sun, MD[2], Jennifer Baker, RN[2], Kimberly Carpenter, PhD[1], Scott Compton, PhD[1], Megan Deaver, PhD[1], Lauren Franz, MB, ChB[1], Nicole Heilbron, PhD[1], Brianna Herold, MS[1], Joseph Horrigan, MD[1], Jill Howard, PhD[1], Andrzej Kosinski, PhD[2], Samantha Major, MS[1], Michael Murias, PhD[1], Kristin Page, MD[2], Vinod K. Prasad, MD[2], Maura Sabatos-DeVito, PhD[1], Fred Sanfilippo, MD[3], Linmarie Sikich, MD[1], Ryan Simmons, PhD[2], Allen Song, PhD[2,4], Saritha Vermeer, PhD[1], Barbara Waters-Pick, MD[2], Jesse Troy, PhD[2], and Joanne Kurtzberg, MD[2]

Duke ACT: Trial Design



180 children, ages 2-7 years
6 month f/u completed OCT 2018, 12 month FEB 2019

Safety Outcomes

- There were no significant SAEs observed during the study.
- Infusion reactions occurred in 4/61 children in the placebo group, 2/56 children in the autologous cord blood group and 3/63 children in the allogeneic cord blood group. None were serious and all resolved with additional treatment with Benadryl, albuterol and or a second dose of solumedrol.
- Many parents reported mild and transient anxiety or other psychiatric symptoms in their children post infusion (27/61 placebo; 22/56 autologous; 30/63 allogeneic).

Baseline Characteristics

Despite our efforts to enroll 143 children with normal cognitive function, only 101 children with NVIQ >70 were accrued to the study. There was also an imbalance in the distribution of children with higher function between the autologous and allogeneic cord blood cohorts with the allogeneic group enrolling ~17% fewer children with a NVIQ >70 compared to the placebo or autologous cord blood cohort. In addition, the children treated with allogeneic cord blood received a significantly higher cell dose than the children receiving an autologous cord blood infusion (38.45 million cells/kg versus 26.88 million cells/kg)

	Randomized Group		Cord Blood Infused	
	Cord_Blood (N=119)	Placebo (N=57)	Autologous Cord Blood (N=56)	Allogeneic Cord Blood (N=63)
Patient Characteristics				
Sex, N (%)				
Female	21 (17.6)	15 (26.3)	9 (16.1)	12 (19.0)
Male	98 (82.4)	42 (73.7)	47 (83.9)	51 (81.0)
Age, years, median (range)	5.30 (2.39, 8.00)	5.22 (2.31, 7.99)	5.09 (2.74, 7.99)	5.33 (2.39, 8.00)
Race, N (%)				
Non-White	24 (20.2)	15 (26.3)	13 (23.2)	11 (17.5)
White	95 (79.8)	42 (73.7)	43 (76.8)	52 (82.5)
Full Scale IQ, median (range)	67 (30, 115)	70 (31, 122)	76.5 (37, 110)	62 (30, 115)
Non-verbal IQ, N (%)				
< 55[a]	32 (26.9)	17 (29.8)	10 (17.9)	22 (34.9)
< 70[b]	53 (44.5)	22 (38.6)	20 (35.7)	33 (52.4)
> 70	66 (55.5)	35 (61.4)	36 (64.3)	30 (47.6)
ADOS Severity, median (range)	19 (3, 27)	20 (7, 28)	18 (3, 26)	20 (7, 27)
Cord Blood Characteristics				
TNC x 10e6, median (range)	730.50 (278.69, 1455.50)	N/A	583.23 (278.69, 1283.80)	883.00 (502.60, 1455.50)
TNC x 10e6/kg infused, median (range)	35.39 (15.14, 64.16)	N/A	26.88 (15.14, 57.57)	38.45 (20.68, 64.16)
CD34+ x 10e6, median (range)	1.08 (0.13, 6.56)	N/A	0.70 (0.13, 4.30)	1.53 (0.13, 6.56)
CD34+ x 10e6/kg infused, median (range)	0.05 (0.01, 0.29)	N/A	0.03 (0.01, 0.27)	0.07 (0.01, 0.29)
CFU x 10e5, median (range)	43.11 (0.00, 1455.60)	N/A	23.53 (0.00, 111.70)	63.85 (0.00, 1455.60)
CFU x 10e5/kg infused, median (range)	2.26 (0.00, 61.94)	N/A	1.15 (0.00, 5.53)	2.83 (0.00, 61.94)
Viability %, median (range)	95 (74, 100)	N/A	95.5 (75, 100)	95 (74, 100)
Sterility, N (%)				
No Growth	119 (100.0)	N/A	56 (100.0)	63 (100.0)

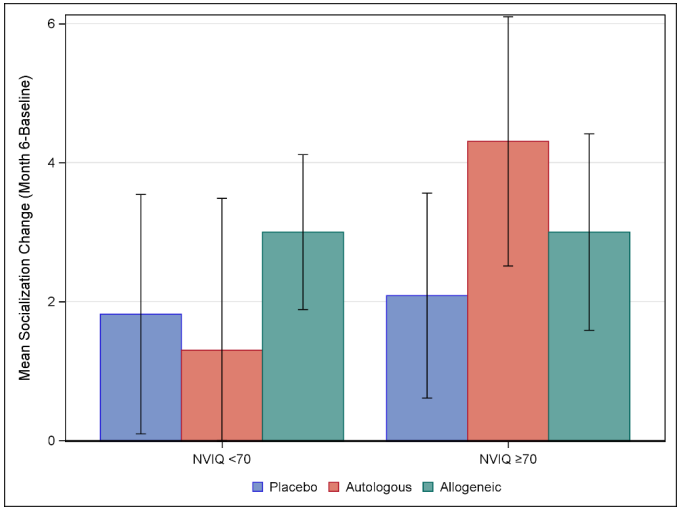
IQ = Intelligence Quotient. NVIQ = Non-verbal IQ. ADOS = Autism Diagnostic Observation Schedule.

[a] Randomization strata. One individual with NVIQ=58 was incorrectly randomized to <55 strata.

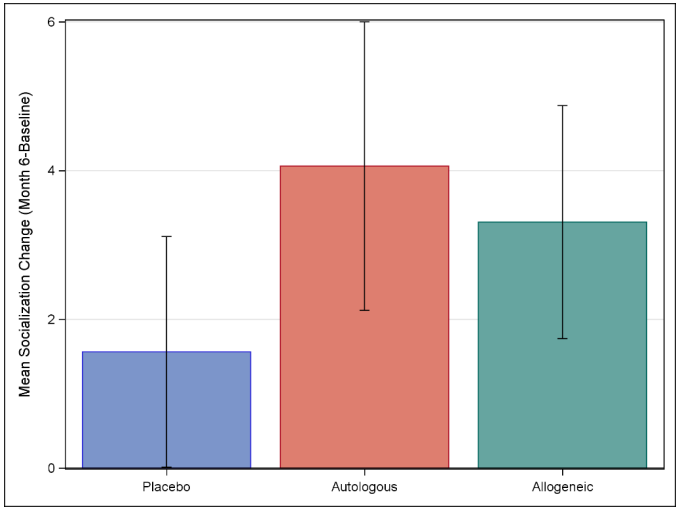
[b] Threshold for intellectual disability.

Mean Change in VABS-3 Socialization Standard Score at Month 6

Everyone



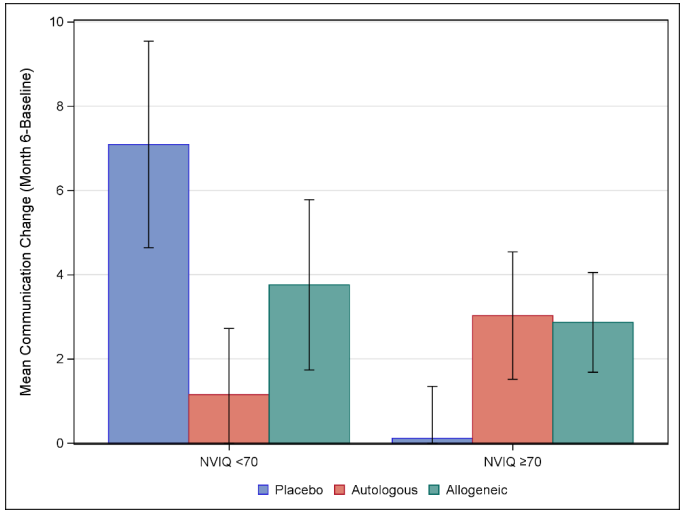
Age 4-7, NVIQ ≥70



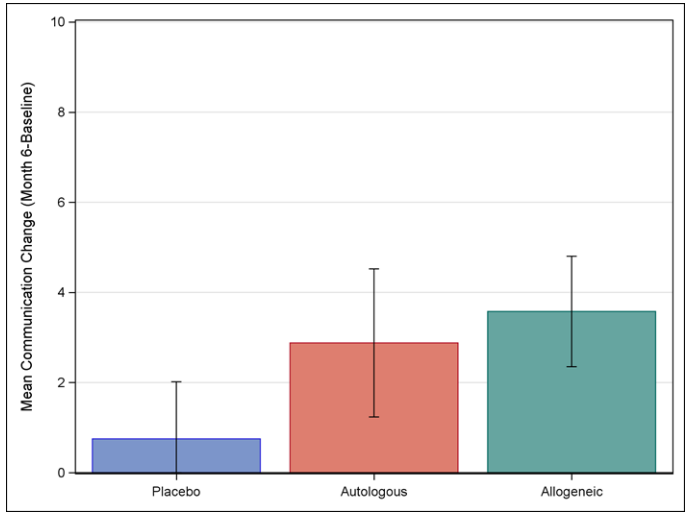
Response in the Placebo group (left panel) was higher than expected, even in higher functioning children (NVIQ ≥= 70). Group differences are more pronounced in older and higher-functioning children (right panel).

Mean change in VABS-3 Communication Standard Score at month 6

Everyone

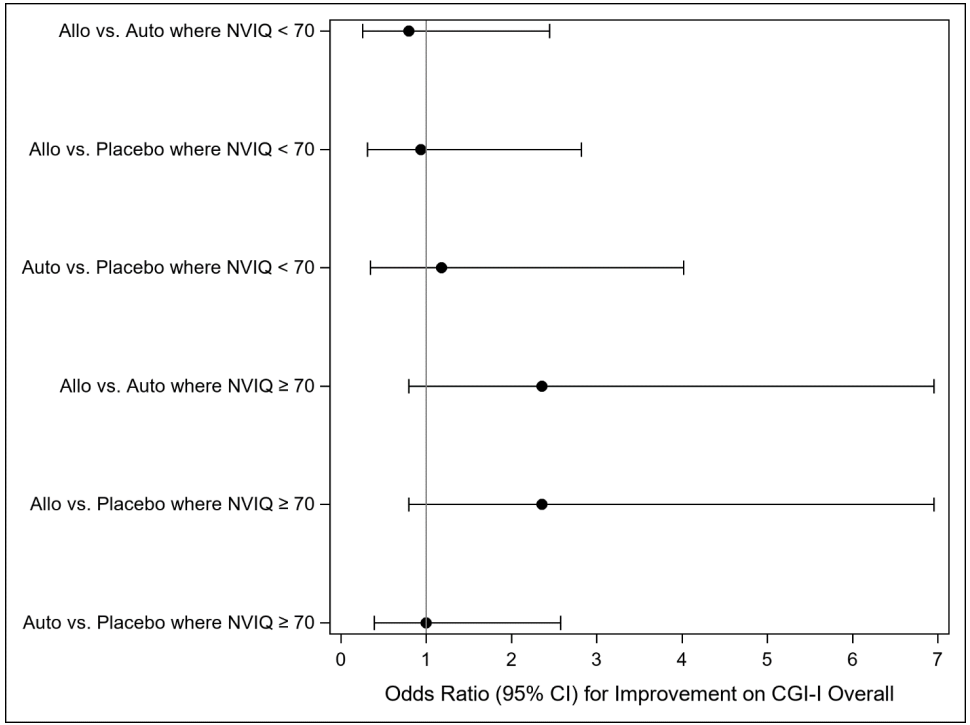


Age 4-7, NVIQ ≥70



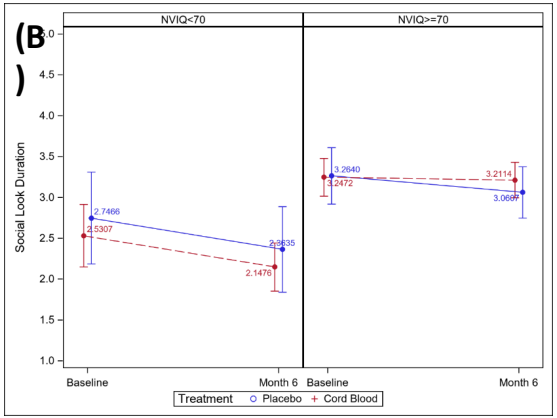
Response to placebo was especially high in children with intellectual disability (NVIQ < 70; left panel). Older children with higher cognitive function were less likely to respond to placebo (right panel).

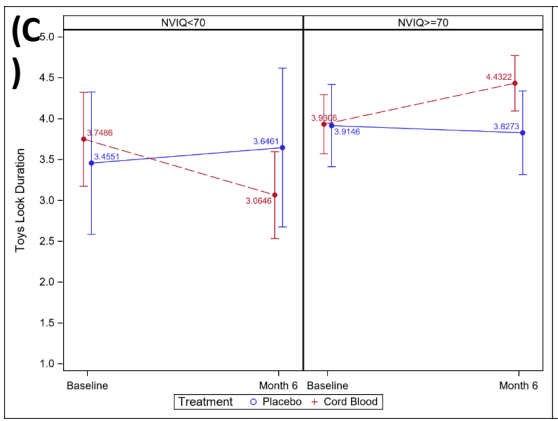
Results of Logistic Regression Comparing Odds of Clinician-Assessed Improvement by Type of Cord Blood and Non-Verbal Intelligence Quotient

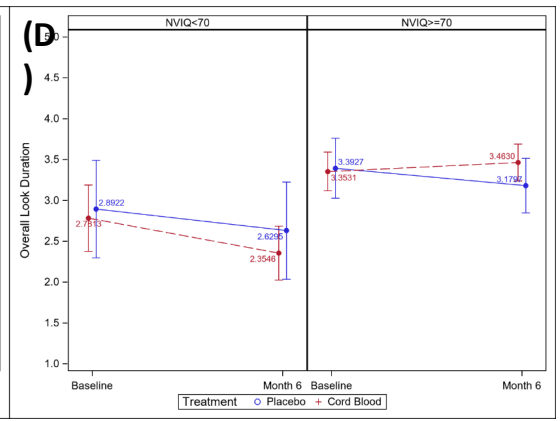


The Clinical Global Impression – Improvement (CGI-I) is a clinician-assessed 7-level ordinal scale. In this analysis, Improvement is defined as Very Much Improved, Much Improved, or Slightly Improved. No improvement is defined as No Change, Slightly Worse, or Much Worse. In this analysis, recipients of allogeneic cord blood without intellectual disability improved compared to placebo while recipients of autologous cord blood did not.

Results of Eye Tracking Analyses

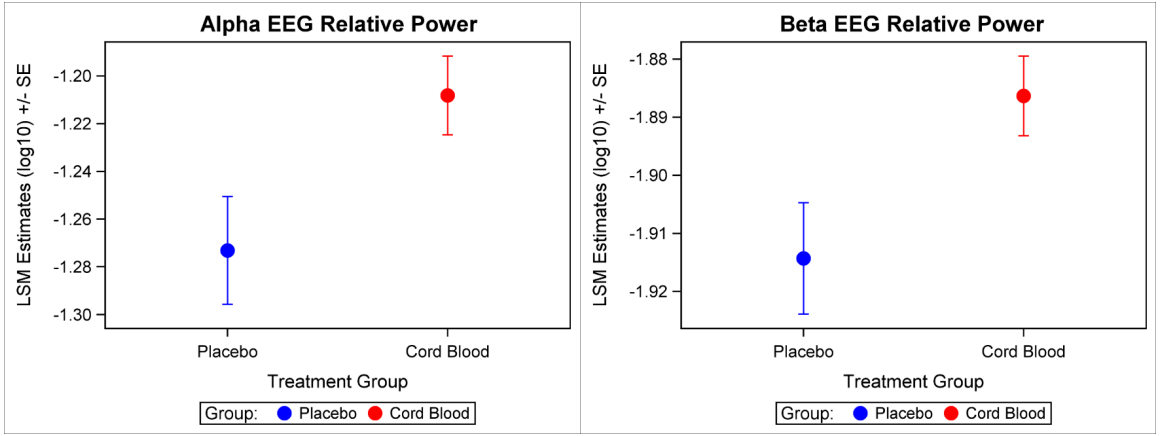






Panel (A) shows a still image from the video, illustrating the potential targets the viewer can look at, e.g., toys and actress during different experimental conditions (Dyadic Bid: the actress attempts to get the viewer's attention; Moving Toys: the toys begin to move and make noise). Plots show the mean look duration and 95% confidence interval for the mean at Baseline and Month 6 by assigned treatment and baseline non-verbal intelligence quotient (NVIQ). Panel (B) shows Look duration at media during the Dyadic Bid Panel (C) shows look duration at the media during the Moving Toys Condition. Panel (C) shows look duration averaged over the Dyadic Bid and Moving Toys conditions.

EEG Spectral Power Outcomes at 6 Months for Participants with NVIQ ≥ 70



NVIQ=non-verbal intelligence quotient measured at baseline. Relative EEG spectral power outcomes are based on analysis of covariance (ANCOVA) where the 6-month scores shown were regressed on the baseline value. (Top) Relative alpha EEG power (posterior region, Toys video) and (Bottom) Relative Beta1 EEG power (all brain regions, Social video).

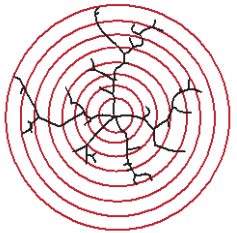
Conclusions

- Primary analysis of the whole study population did not show a benefit of cord blood over placebo on the VABS-3 Socialization Scale
  - There was a flaw in study design, sample size didn't achieve goal (101 vs 143)
- For children with no intellectual disability (NVIQ $\geq$70):
  - improvements in communication (VABS-3 Communication Scale), attention (eye tracking), and increased alpha and beta EEG power.
  - Children receiving allogeneic cord blood showed improvement on the Clinical Global Impression-Improvement scale, compared to placebo.
- Notably, the high expectancy effect in the placebo arm and the larger number or participants with intellectual disability, compromised the interpretation of the results of the study.

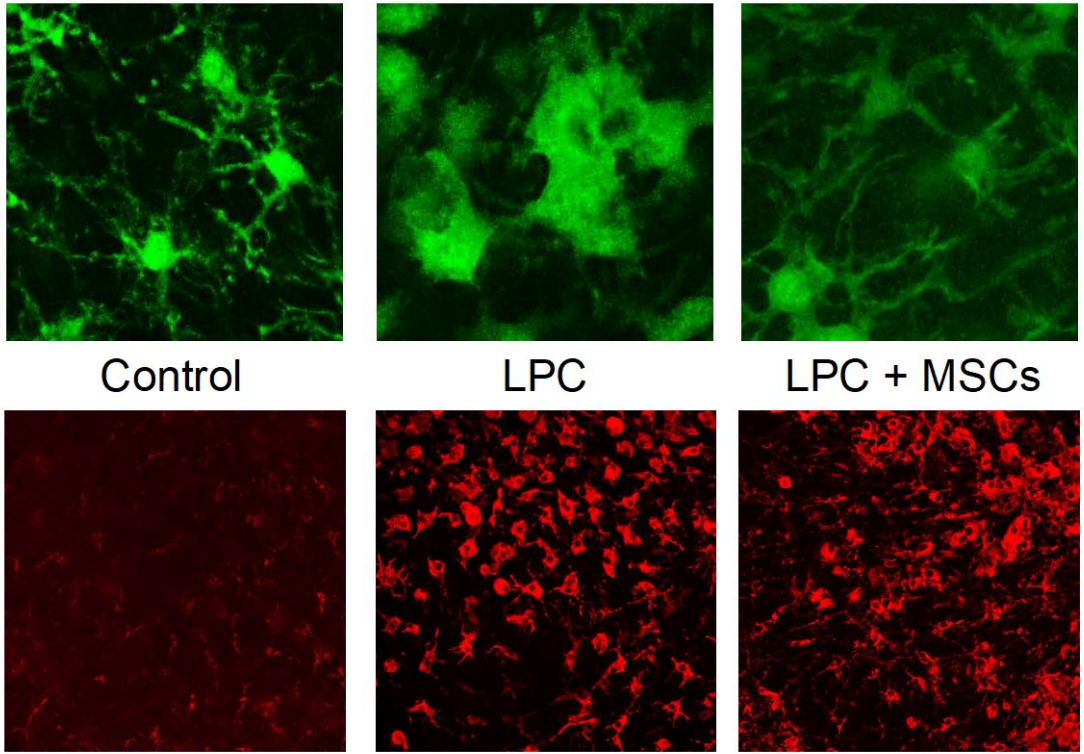
Manufacturing of allogeneic CT MSCs

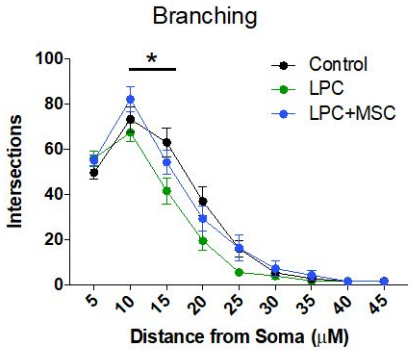
- Donated CB, maternal consent
- Healthy term male baby delivered by CS
- Tissue digestion with 4 GMP grade enzymes
- Plate to P0, P1, P2, Cryopreserve
- Thaw, dilute, infuse
- Characterization, sterility, endotoxin
- Potency
  - Suppression of a 3rd party MLC
  - Suppression of microglial activation

hCT-MSCs in children with ASD



MSCs and CB CD14 cells Inhibit Microglial Activation





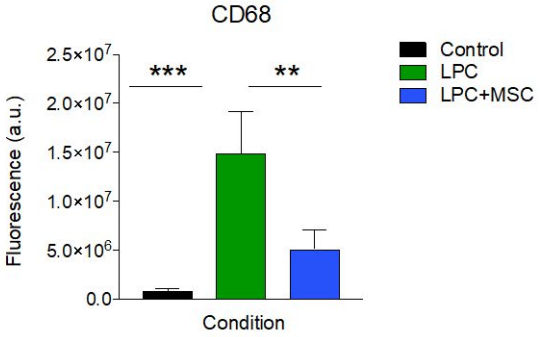
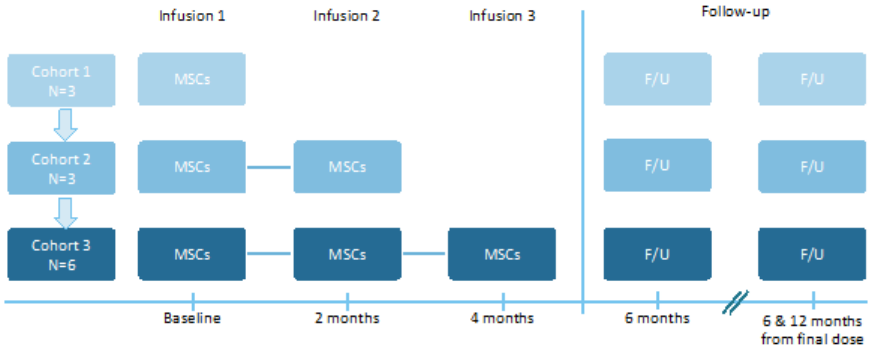
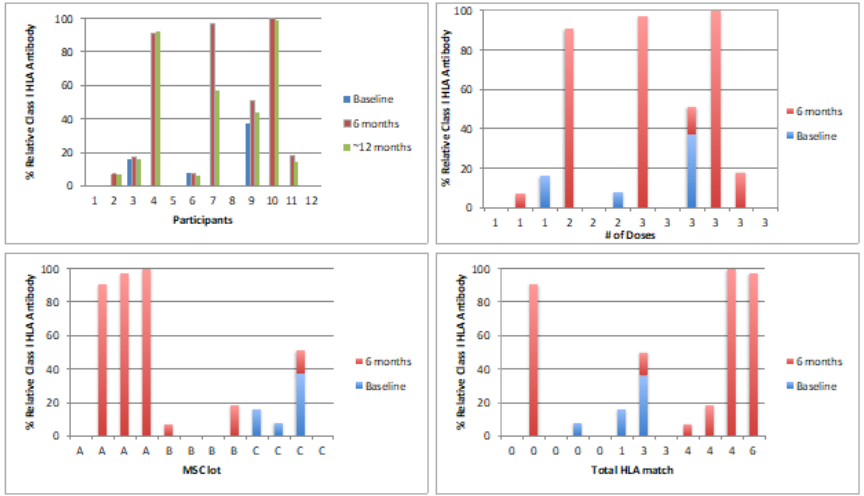


Figure 1: Study Design



12 patients
27 doses
3 dosing cohorts
3 donors
2M cells/kg/dose

Figure 3: Class I anti-HLA Antibodies. Panel A: Presence of Class I HLA antibodies at baseline, 6 months, and >12 months by participant (≥12 month data not available for participants 3, 4, 11, 12). Panel B: Class I HLA antibodies and baseline and 6 months by number of hCT-MSC doses. Panel C: Class I HLA antibodies at baseline and 6 months by lot of hCT-MSC. Panel D: Class I HLA antibodies by HLA match (at HLA-A, B, C, DRB1) between hCT-MSC donor and recipient.



HLA type of each lot:

LOT	A_1	A_2	B_1	B_2	DRB1_1	DRB1_2
A	A*02:01:01G	A*25:01:01G	B*07:02:01G	B*44:02:01G	04:AJEAD	11:ANMAJ
B	A*01:01:01G	A*29:02:01G	B*08:01:01G	B*44:03:01G	03:ANCAB	15:ANUAP
C	A*02:01:01G	A*03:01:01G	B*07:02:01G	B*07:02:01G	01:03	15:ANTZM

Phase 1 MSC study – 6 month data

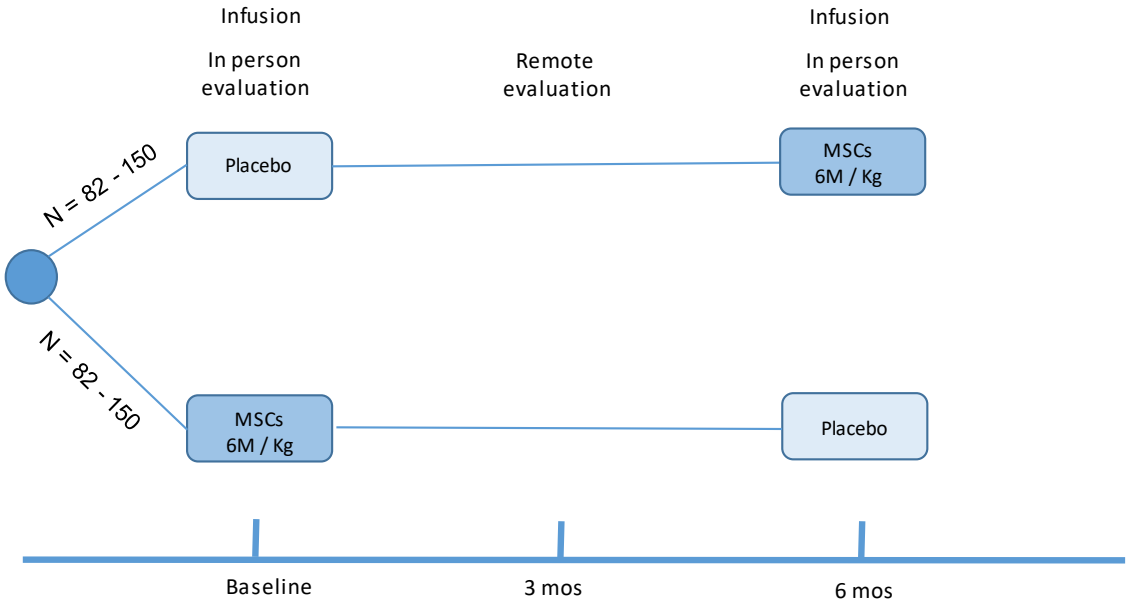
ID	Dose	Sex	IQ	VABS*	PDDBI	CGI	Improvement
1	1	M	62	-2	-	Min	1
2	1	M	68	4	6	Min	2
3	1	M	45	22	-22	Min	3
4	2	F	59	0	-6	Much	2
5	2	M	40	-10	-1	No	0
6	2	M	36	8	-22	Min	3
7	3	M	42	-2	0	No	0
8	3	M	54	-8	-4	No	1
9	3	M	71	-3	6	Min	1
10	3	M	82	19	-20	Min	3
11	3	F	59	4	-7	Min	3
12	3	F	95	7	-2	Min	3

* Clinically significant improvement $\geq$ 3 points.

- ***58% of patients (7/12) showed improvement on at least 2/3 measures.***
  - 42% (5/12) showed improvement on 3/3 measures.
  - 16% (2/12) showed improvement on 2/3 measures.
  - No clear dose effect, although there are too few patients to determine this definitively.

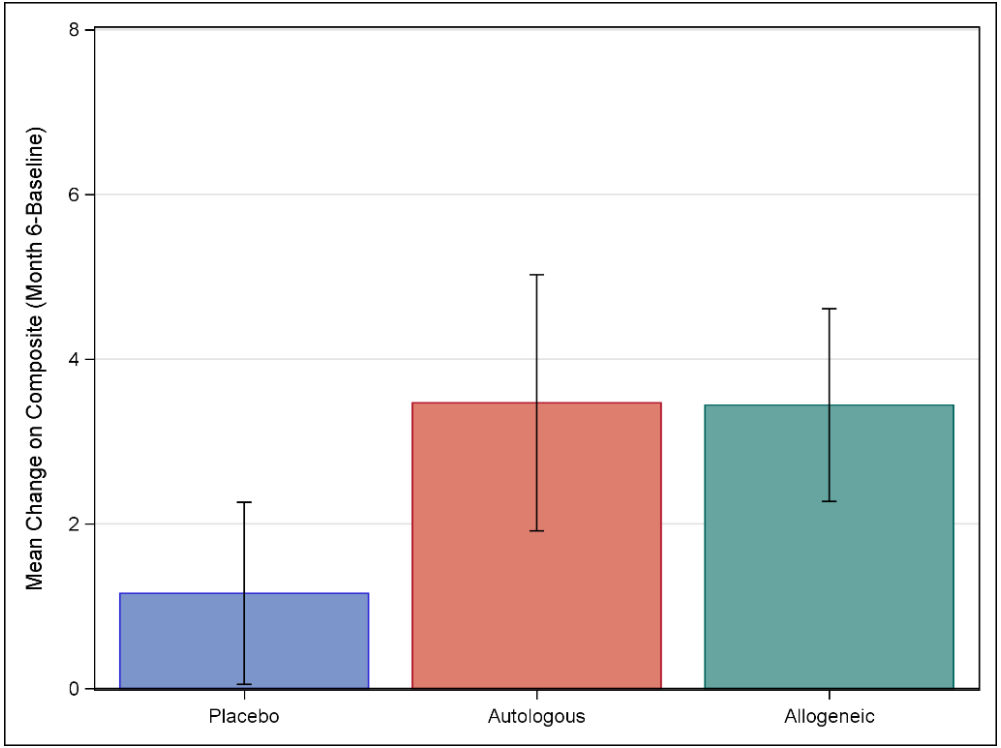
IMPACT ASD: A randomized, blinded, placebo-controlled phase 2 study of hCT-MSCs in children with autism spectrum disorder

- 164-300 subjects
- Ages 4-11 years
- FS IQ ≥70
- Blinded crossover design
- hCT-MSC 6M/kg
- Primary Endpoint
  - Mean of change of VABS-3 SS + CS (composit)
- Secondary Endpoints
  - Eye tracking
  - EEG
  - CGI
  - PDDBI



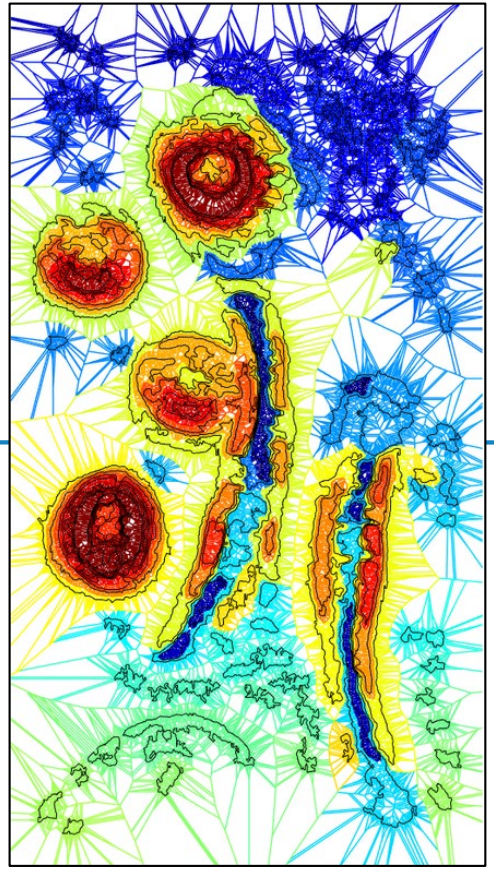
Primary Endpoint for IMPACT Study: Composite of Mean Change in VABS-3 Socialization and Communication Standard Scores

Age 4-7, NVIQ ≥ 70



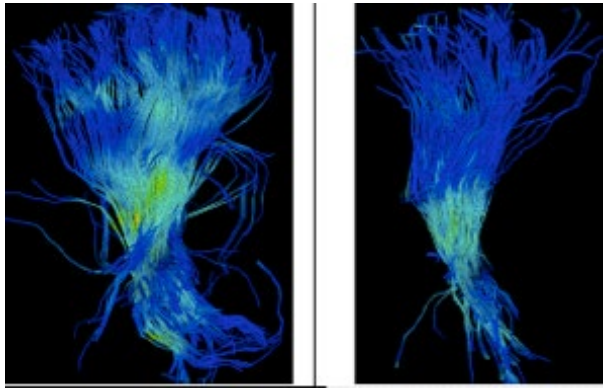
DUOC-01:
A CB-Derived Cellular Therapy

to remyelinate the brain

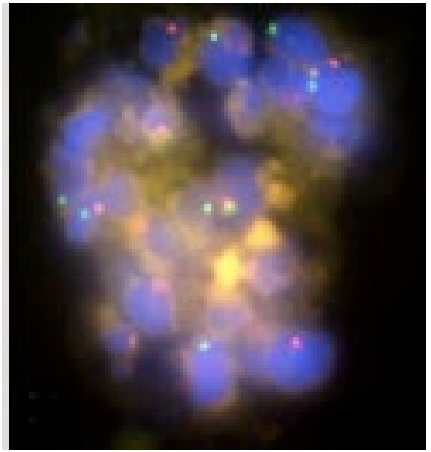


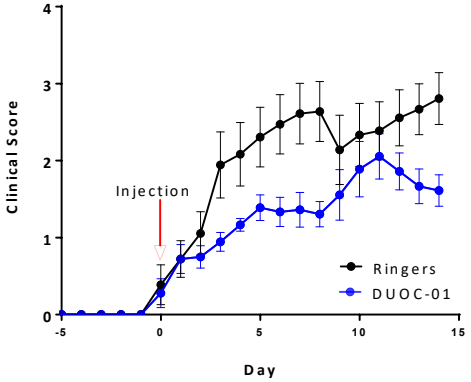
<u>UCBT for EIKD:</u>

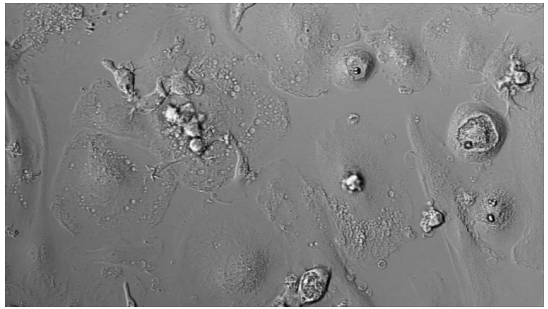
Functional Outcomes vary with best outcomes in babies transplanted in the first month of life

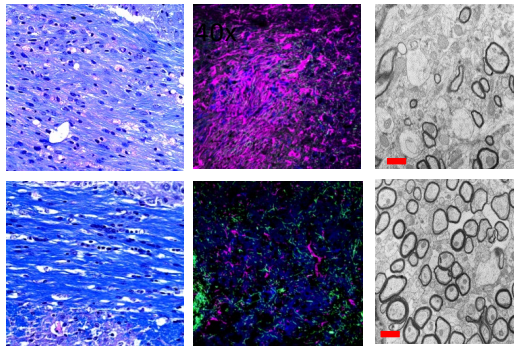


DUOC was invented after we observed donor cells engrafting in the brain after CBT



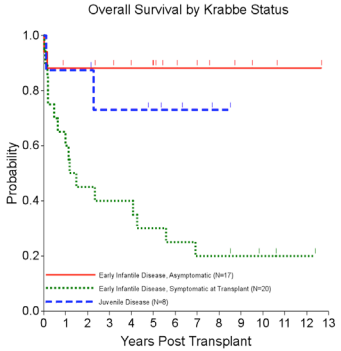


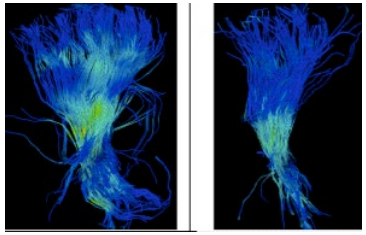


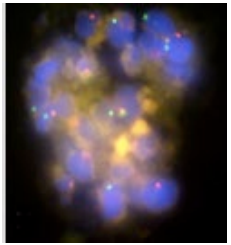


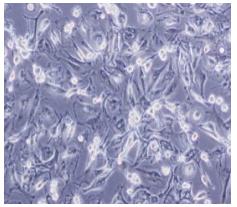
- Enzyme replacement
- "Clean up"
- Cytokine secretion
  - Modulate inflammation
  - [IL10, IL6, TGF-beta
  - Inhibits cellular infiltration
- Drives oligodendrocyte proliferation
- Promotes myelination

DUOC-01, a bridging therapy augmenting UCBT in LSDs

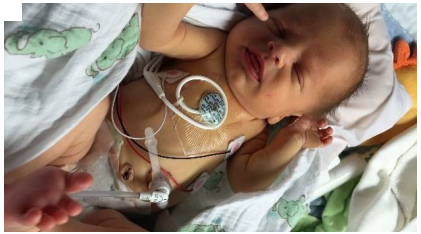








Day 0 IV



OUTCOMES

Day 28 IT

Begin D7-21

- **28 Patients treated with DUOC-01**

- **2 reversible reactions in 2/3 patients receiving different donor DUOC cells**
- **IND amended to add HC with IT dose. No reactions since**

Hard to assess efficacy in these diseases which have improvement and variable courses post transplant

- **Exploring MOA in EAE model of MS**
- **Planning clinical trials in adult MS and other demyelinating diseases**

Conclusions and Future Plans

- **Conclusions:**
- **CB, both autologous and allogeneic, show excellent safety profiles and suggestions of efficacy in Phase 1 and 2 clinical trials in children with brain injury.**
- **The CB monocytes appear to be the active cells in CB, a heterogeneous cell product**
- **Additional, well designed Phase 3 studies, will be required to confirm efficacy and to obtain regulatory approvals**
- **CT-MSCs modulate neuroinflammation and are undergoing testing in children with ASD**
- **These therapies have the potential to treat diseases with unmet needs and to change human lives**

- **Future Plans**
- **ASD: Complete IMPACT**
  - **Small trials in toddlers and AYAs**
- **CP: Conduct a multicenter Phase 3 trial with cord blood**
- **HIE: Conduct a multicenter Phase 3 trial with cord blood/MSC**
- **DUOC: Conduct a Phase 1a trial in adults with MS**
- **Miles: MSC source comparison in OA Knee**
- **Continue trials in COVID-ARDS, COVID MIS-C**

Acknowledgements

- Patients & Families
- The Marcus Center for Cellular Cures (MC^3)
  - Joanne Kurtzberg MD
  - Geraldine Dawson PhD
  - Jesse Troy PhD
  - Amanda Parrish PhD
  - Jennifer Baker
  - Andrew Balber PhD
  - Anthony Filiano PhD
  - Arjun Saha PhD
  - Paula Scotland, PhD
  - Pamela Noldner
  - Lynn Cheatham
  - Ann Kaestner, MT
- Cord Blood Banks
- Funding Support
  - The Marcus Foundation
  - The Robertson Foundation
  - The Dana Foundation
  - Cure CP
  - Perkin Elmer
- Study Teams
  - Joanne Kurtzberg MD
  - Geraldine Dawson PhD
  - Jessica Sun MD
  - Michael Cotton, MD
  - Kristin Page MD
  - Vinod Prasad MD
  - Jesse Troy PhD
  - Mohamad Mikati MD
  - Gordon Worley MD
  - Allen Song PhD
  - Joan Jasien MD
  - Katie Gustafson PhD
  - Laura Case DPT
  - Julie Coats PT
  - Colleen McLaughlin CPNP
  - Tara West PNP
  - Rachel Hollowell PNP
  - Natalie Skergen PNP
  - Jayne Cash RN
  - Kerry Hoyle RN
  - Sydney Crane RN
  - Barbara Waters-Pick, MT
  - The EMMES Corporation & RTI
  - Duke STCL
  - Carolinas Cord Blood Bank
  - Robertson GMP Manufacturing Lab

"It takes a Village"