AN OPEN LABEL PHASE I STUDY OF hCT-MSC, AN UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELL PRODUCT IN YOUNG CHILDREN WITH AUTISM SPECTRUM DISORDER

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AN OPEN LABEL PHASE I STUDY OF hCT-MSC, AN UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELL PRODUCT IN YOUNG CHILDREN WITH AUTISM SPECTRUM DISORDER

I agree to conduct and supervise this clinical study in accordance with the design and specific provisions of this protocol; modifications to the study or protocol are acceptable only with a mutually agreed upon protocol amendment except when necessary to protect the safety of participants. I agree to await IRB approval for the protocol and informed consent before initiating the study, to obtain informed consent from participants prior to their enrollment in the study. I agree to report to responsible regulatory agencies and the IRB (when necessary) adverse events that occur in the course of this investigation. I agree to maintain accurate and adequate records in the case report forms as required by this protocol and maintain those records for the period of time required. I will make the study documentation available for safety oversight committee review and/or for other inspections as required. I agree to maintain study documentation for the period of time required. I agree to comply with all other requirements regarding the obligations of clinical investigators according to FDA regulations and guidance. I agree to ensure that all people assisting in the conduct of this study are informed in meeting the above commitments.

_____
(Investigator's printed name)

_____
(Investigator's signed name)

PROTOCOL SUMMARY

Protocol Number:	
Title:	AN OPEN LABEL PHASE I STUDY OF hCT-MSC, AN UMBILICAL CORD TISSUE-DERIVED MESENCHYMAL STROMAL CELL PRODUCT IN YOUNG CHILDREN WITH AUTISM SPECTRUM DISORDER
Study Phase:	I
Study Site:	Single site; Duke University, Durham NC
Study Therapy, Dosage, and Route of Administration:	Umbilical Cord Tissue Mesenchymal Stromal Cells, isolated and expanded from umbilical cord donors. One dose of $2x10^6$/kg administered intravenously.
Objectives:	To determine the safety of a single intravenous infusion of hCT-MSC in very young children with ASD.
Research Participant Population:	Twelve toddlers, ages 18 months to <4 years (3 years and 364 days), with an established diagnosis of autism spectrum disorder (ASD)
Study Design:	Prospective
Safety Assessments/Endpoints:	1. Incidence of infusion reactions 2. Incidence of infections 3. Incidence of clinically significant anti-HLA antibody formation

ABBREVIATIONS

ADOS	Autism Diagnostic Observation Schedule
AE	Adverse Event
ASD	Autism Spectrum Disorder
BOSA	Brief Observation of Symptoms of Autism
CB	Human Umbilical Cord Blood
CDI	Communication Development Inventories
CFR	Code of Federal Regulations
CGI	Clinical Global Impression
CMP	Complete Metabolic Panel
CMV	Cytomegalovirus
CNS	Central Nervous System
CNV	Copy Number Variation
CRF	Case Report Form
CVA	Computer Vision Analysis
CTCAE	Common Terminology Criteria for Adverse Events
DAYC-2	Developmental Assessment of Young Children-Second Edition
DSMB	Data Safety Monitoring Board
DMSO	Dimethyl Sulfoxide
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EDC	Electronic Data Capture
EEG	Electroencephalography
EOWPVT	Expressive One Word Picture Vocabulary Test
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Federal Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GvHD	Graft versus Host Disease
HBV	Hepatitis B Virus
hCT-MSC	Human Umbilical Cord Tissue-derived Mesenchymal Stromal Cells
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRPP	Human Research Protections Program
HSCT	Hematopoietic Stem Cell Transplantation
HTLV	Human T-lymphotropic Virus
ICH	International Council for Harmonisation
IRB	Institutional Review Board
ISBT	International Society for Blood Transfusion
IV	Intravenous
IVIG	Intravenous Immunoglobulin
JERI	Joint Engagement Rating Inventory
MBHQ	Medical/Behavioral History Questionnaire
mITT	Modified Intention to Treat
MSC	Mesenchymal Stromal Cell
NMDP	National Marrow Donor Program
NSAID	Non-steroidal Anti-inflammatory Drug
PCIT	Parent-Child Interaction Task
PDDBI	Pervasive Developmental Disorder Behavior Inventory
PET	Positron Emission Test
PRA	Panel Reactive Antibody
RBS-EC	Repetitive Behavior Scale for Early Childhood
S2K	Sense to Know
SAE	Serious Adverse Event
SNP	Single-nucleotide Polymorphism
TNC	Total Nucleated Cells
VABS	Vineland Adaptive Behavior Scales
WBC	White Blood Cell

1.0 PURPOSE

The purpose of this Open Label Phase I study is to determine the feasibility and safety of treatment via a single intravenous infusion of human umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC) in very young children with autism spectrum disorder (ASD).

2.0 BACKGROUND AND HYPOTHESIS

2.1 AUTISM SPECTRUM DISORDER

Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with onset early in life. It is characterized by impairments in social communication and the presence of repetitive behaviors and a restricted range of activities.[1] ASD is 4 to 5 times more prevalent in boys, with approximately 1 in 59 children in the United States diagnosed with ASD.[2] ASD is often accompanied by intellectual disability and is usually a chronic, disabling disorder that compromises the full potential of the affected individual. The majority of individuals with ASD are not able to live independently and require lifelong support or accommodations. Accordingly, the lifetime cost of supporting an individual with ASD is estimated to be $1.4 million. The cost is $2.4 million for those who also have an intellectual disability.[3]

Early behavioral intervention leads to improved outcomes in individuals with ASD.[4] Other treatments include occupational and speech therapy, and specialized educational and vocational support. Despite receiving high quality early behavioral intervention and related therapies, many individuals with ASD continue to have significant impairments, with a substantial percentage not acquiring speech. All of the currently available medical treatments, such as psychotropic medications, are intended to ameliorate associated co-morbid symptoms, such as irritability, but do not address core symptoms, such as the ability to socially interact and communicate. In light of this, there is a large unmet need for effective medical treatments for ASD that address core autism symptoms.

The etiology of ASD is complex and includes both genetic and environmental factors. In recent years, genetic sequencing and analysis have identified several de novo mutations, copy number variants (CNV), and single-nucleotide polymorphisms (SNP) that are associated with an increased risk of ASD. However, it is estimated the specific genetic cause can only be identified in less than 20% of cases, with the remaining causes likely being caused by an interaction between multiple genetic and environmental risk factors.[5] Several environmental factors have also been associated with an increased risk of ASD, including prematurity, birth complications, maternal teratogen exposure, environmental toxins, and advanced paternal age. Finally, inflammation, particularly neuroinflammation, and immune dysregulation have been implicated in the etiology of ASD. Therefore, immunomodulatory therapies may have a role in the treatment of children with ASD.

2.2 IMMUNE DYSREGULATION IN ASD

A role of immune activation and/or dysregulation in the etiology of ASD is supported by several different observations. Multiple epidemiologic studies have demonstrated an increased rate of ASD in children born to mothers who had an infection during pregnancy. This association has been consistent across countries and time periods, having been reported as early as the 1970s after a rubella pandemic in the United States[6] and throughout the decades in Denmark,[7] Sweden, and other countries. Maternal fever, antibiotic treatment, elevated levels of inflammatory markers, and infection with several different microbes have been associated with increased risk for the development of ASD in the child, indicating that

the immune response to infection is likely to be more causative than the infectious agent itself. Accordingly, animal models have yielded offspring with ASD phenotypes by inducing immune activation in the pregnant mothers.[8,9]

Potential pathophysiologic mechanisms include alterations in maternal-fetal immune tolerance (via maternal antibodies and/or cellular immunity) and fetal brain inflammation, which lead to changes in brain cytokine profiles and microglial activation that may be detrimental to neurodevelopment. Evidence of increased numbers of microglia and increased microglial activation in ASD has been obtained via autopsy studies, positron emission tomography (PET) brain imaging, and animal models. In addition, abnormal functioning in aspects of the immune system in the brain (such as microglia that are tasked with providing support to neuronal synapses) has been described.[10,11] Increased plasma cytokine levels, upregulated genes associated with microglial activation, localized inflammation and pathological astrocyte activation have also been associated with ASD.[12] Of note, many cytokines and molecules classically associated with immune regulation are now recognized as also playing a role in normal neurodevelopment. This dual functionality may prove to be an important link in the association of immune-related changes and abnormal neurodevelopment in ASD.

2.3 SYNAPTIC DYSFUNCTION IN ASD

Synapses are points of communication between neurons, allowing the organized passage of information via electrical and chemical signaling. While there is a period of intensified synaptogenesis early in development, synapses retain plasticity throughout life, enabling, for example, learning and memory. Normal synaptic development and maintenance are essential to proper neuronal function, and abnormalities in either process have been associated with multiple neurodevelopmental conditions, including ASD. Mutations in genes encoding synaptic proteins have been implicated in ASD. Additionally, human and animal studies have demonstrated a reduction in the size, number, and morphology of dendritic spines and an increase in immature spine morphology in ASD and autism related disorders.[13] It is also likely that environmental factors may also influence synaptic changes. These alterations may lead to problematic neuronal connectivity, such as large-range under-connectivity and short-range over-connectivity.[14,15]

Altogether, these observations suggest that both environmental and genetic risk factors may contribute to the development of ASD by causing immune dysregulation and/or abnormal neuronal connectivity that adversely affect normal brain development and cause core symptomatology observed in individuals with ASD.

2.4 MESENCHYMAL STROMAL CELLS (MSCs)

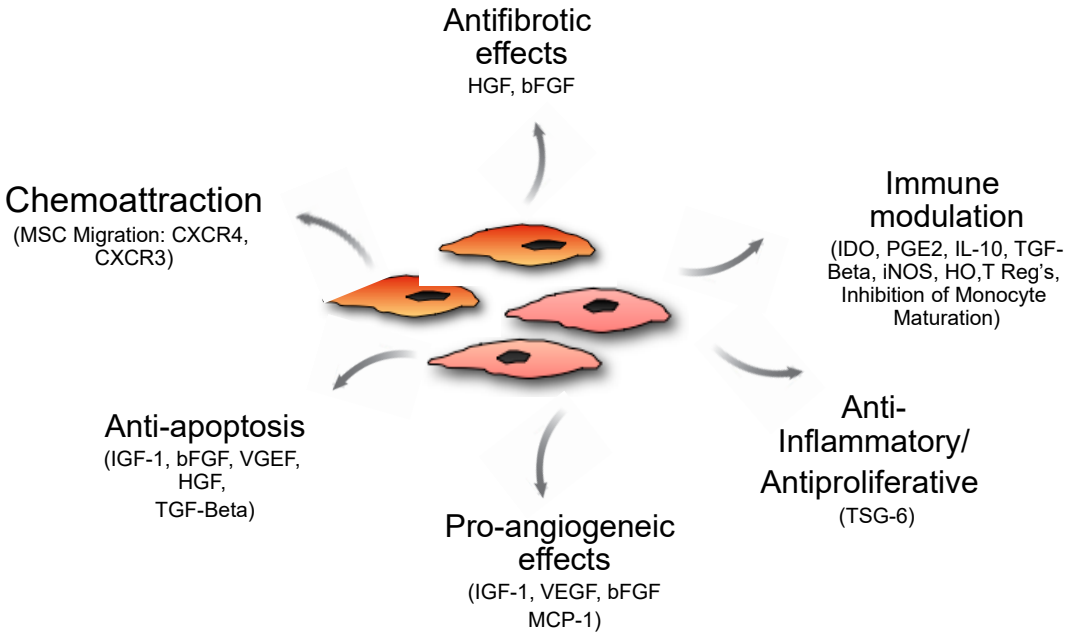
hCT-MSC is a third party, allogeneic, human mesenchymal stromal cell (MSC) product manufactured from donated umbilical cord tissue, after written informed consent for donation and commercialization, harvested from discarded birthing tissues, that is digested, expanded for two passages in culture in xeno free media, harvested, cryopreserved, and stored in the vapor phase of liquid nitrogen until use. The umbilical cord tissue is donated by healthy mothers delivering full term babies via Cesarean Section after written informed consent. The cells are cryopreserved and stored in the Robertson CT2 GMP laboratory (2400 Pratt Street, Duke University, Durham, NC).

Mesenchymal stromal cells (MSCs):

MSCs are a heterogeneous group of undifferentiated, multipotent cells that can be isolated from several different tissues including bone marrow, adipose tissue, and birth tissues (umbilical cord blood, umbilical cord tissue, amniotic fluid, placenta). While MSCs can give rise

to mesodermal tissue types including bone, cartilage, and fat, their primary mechanism of action is thought to result from paracrine effects on immunomodulation, antiapoptosis, angiogenesis, support of the grown and differentiation of local stem and progenitor cells, antifibrosis, and chemoattraction (figure 1).[16] MSCs have demonstrated a multitude of immunomodulatory effects on both humoral and cell-mediated immune responses. These include, but are not limited to, inhibiting B-, T-, NK, dendritic-cell, and microglial proliferation, decreasing pro-inflammatory cytokine production, and blocking neutrophil recruitment.

Figure 1: Paracrine effects of MSCs, including a subset of factors secreted by cultured MSCs. (Figure adapted from Singer, Annu. Rev. Pathol. Mech. Dis., 2011.[16])



Despite their ability to modulate the immune response, MSCs themselves have low immunogenicity. MSCs express low levels of MHC class I molecules on their surface and lack expression of MHC class II and several costimulatory molecules. This allows MSCs to be used in the allogeneic setting, without the need for donor-recipient HLA matching typical of other cellular therapies or transplantation. In fact, in a review of 13 human studies of intravenous allogeneic MSC administration, there were no reports of infusional toxicity[17] or longer term SAEs, supporting the notion that MSCs are "immune-privileged" and can avoid immunological allorecognition. When utilized as a therapeutic cell, it is widely accepted that MSCs exert effects via paracrine and trophic signaling. They do not engraft in the recipient.

Safety of MSCs:

MSCs manufactured from bone marrow and adipose tissue have been studied in hundreds of clinical trials worldwide involving thousands of individuals and a wide variety of human conditions. Their safety has been repeatedly demonstrated and is summarized in a 2012 systematic review and meta-analysis[17] of 36 clinical trials in 14 countries using MSCs in over 1,000 recipients with cardiovascular, neurological, oncologic, metabolic, gastrointestinal, and post-transplant conditions. Based on a combination of randomized controlled trials and uncontrolled studies with follow-up periods of weeks to up to 5 years, there was no association

between MSC treatment and acute infusional toxicity, organ system complications, infection, death, or malignancy. The only side effect associated with MSC treatment was transient fever, which did not cause any long-term sequelae. Importantly, no malignancies were reported in patients without a prior history of cancer.

After intravenous administration, approximately 70% of infused MSCs are engulfed by lung macrophages. While there is a theoretical risk of respiratory complications, the only randomized trial in which acute pulmonary reactions have been observed was conducted in patients with chronic ischemic heart failure who received intracoronary MSCs.[18] Thus, this risk seems to be limited to patients whose underlying condition makes them susceptible to developing pulmonary edema. Although a few cardiovascular studies have reported arrhythmias, the incidence was not statistically different from control groups in meta-analysis.[17]

Umbilical cord tissue is an attractive source of MSCs as it is readily available and easily obtained without risk to the maternal or newborn baby donor, is non-controversial, and has a higher proliferative potential than MSCs from other postnatal sources.[19] Numerous preclinical studies have not demonstrated any evidence of tumorigenicity or toxicity of cord tissue-derived MSCs.[20] A summary of early phase clinical trials published in English that utilized cord tissue-derived MSCs is shown in Table 1. Among these 37 studies including 710 patients and at least 1,431 doses of cord tissue-derived MSCs with follow-up ranging from three months up to six years, no severe adverse events were reported. Several more clinical trials of cord-tissue derived MSCs in various disease conditions are underway (clinicaltrials.gov).

Table 1: Umbilical Cord Tissue-Derived MSCs Studied in Humans

	Indication	Report	Ages, yrs (range or mean)	Route of administration	Dose	# of doses (interval)	Duration of f/u (months)	AEs due to CT-MSCs
Cao, 2018[21]	Intrauterine Adhesions	26	36	Intrauterine	$1x10^7$	1	30	none
Xaio, 2018[22]	Spinal Cord Injury	2	29	Spinal cord	$4x10^7$	1	12	none
Riordan, 2018[23]	Multiple Sclerosis	20	41	IV	$2x10^7$	7 (daily)	12	HA, fatigue
Sun, 2018[24]	Kidney Transplant	21	41	IV, intra-arterial	$2\text{-}5x10^6$	2	12	none
Xu, 2018[25]	Severe Aplastic Anemia	24	18	IV	$5x10^5$	1	13	none
Rahyussalim, 2017[26]	Spinal Cord Entrapment, Kidney Failue	1	62	IV, IT	$1.6x10^7$	36	8	none
Park, 2017[27]	Osteoarthritis	6	59	Intra-articular	$1.15\text{-}2x10^7$	1	84	arthralgia, back pain, bladder distension, ↑ antithyroglobulin antibody
Bartolucci, 2017[28]	Heart Failure	15	57	IV	$1x10^6$/kg	1	12	none
Kim, 2017[29]	Atopic Dermatitis	34	29	Subcutaneous	$2.5\text{-}5x10^7$	1	3	none
Wang, 2017[30,31]	Lupus	40	17-54	IV	$1x10^6$/kg	2 (weekly)	12-72	none
Chen, 2016[32]	Osteonecrosis	9	>18	Intra-arterial	$5\text{-}10x10^7$	1	24	none
Li, 2016[33,34]	Autism	20	3-14	IT	$1x10^6$/kg	2 (5-7 days)	6	transient fever (n=4)
Xie, 2016[35]	Hypoxic Ischemic Encephalopathy	12	45-68	IV	$1x10^8$	1	6	none
Wang, 2016[36]	Juvenile Idiopathic Arthritis	10	2-15	IV	$4x10^7$	2 (3 months)	12	none
Chen, 2016[37]	Psoriasis	2	26-35	IV	$1x10^6$/kg	1, 5	48, 60	none
Cai, 2016[38]	Type I Diabetes	21	5-28	Intra-arterial (pancreatic artery)	$1.1x10^6$/kg	1	12	none
Hu, 2016[39]	Type 2 Diabetes	31	42-63	IV	$1x10^6$/kg	2 (monthly)	36	none
Hua, 2016[40]	Spinal Cord Injury	1	25	IT	$1x10^7$	4 (3 days)	12	none
Qin, 2016[41]	Diabetic Foot	28	N/A	Endovascularly	N/A	N/A	3	none
Zhao, 2015[42]	Heart Failure	30	53.2	Intracoronary	NR	1	6	none
Liang, 2015[43]	Radiation Myelitis	1	37	IV, IT	$5.2x10^7$ IV, $1.1x10^7$ IT	1	18	none
Wang, 2015[44]	Cerebral Palsy	16	4-12	IT	$1\text{-}1.5x10^7$	4	6	NR
Li, 2015[45]	Coronary Artery Occlusion	15	N/A	Intracoronary	$3\text{-}5x10^6$	1	24	none

	Indication	Report	Ages, yrs (range or mean)	Route of administration	Dose	# of doses (interval)	Duration of f/u (months)	AEs due to CT-MSCs
Rajput, 2015[46]	Duchenne Muscular Dystrophy	11	5-18	IV & IM	$1x10^6$/kg	4 (monthly)	36	none
Gu, 2015[47]	Lupus Nephritis	58	12-55	IV	$1x10^6$/kg	1	12	none
Pan, 2015[48]	Bone Marrow Necrosis	1	11	Intrabone	$2x10^7$	1	38	none
Wu, 2015[49]	GvHD	24	14-44	IV	$0.5\text{-}1x10^6$/kg	1	1-24	none
Zhu, 2015[50]	Leukemia, undergoing haplo HSCT	25	4-17	IV	$1\text{-}1.4x10^6$/kg	1	3-25	none
Miao, 2015[51]	Neurological disorders	88	2-68	IT	NR	4-6 (5-7 days)	NR	transient HA, fever, back/leg pain
Li, 2015[52]	Becker Muscular Dystrophy	3	6-46	IV	$3\text{-}5x10^7$	1	3	none
Wang, 2015[53]	Hemorrhagic Cystitis	7	11-38	IV	$0.8\text{-}1.6x10^6$/kg	1-3	NR	none
Cheng, 2014[54]	Spinal Cord Injury	10	35.3	Spinal Cord Injection	$2x10^7$	2 (10 days)	6	none
Chang, 2014[55]	Bronchopulmonary Dysplasia	9	10 days	Intratracheal	$1\text{-}3x10^7$	1	3	none
Li, 2014[56]	Multiple Sclerosis	13	42	IV	$4x10^6$/kg	3 (2 weeks)	12	none
Kong, 2014[57]	Type 2 Diabetes	18		IV	N/A	3	6	slight fever
Wang, 2013[58]	Traumatic Brain Injury	20	5-48	IT	$1x10^7$	4 (5-7 days)	6	HA, dizziness
Wang, 2013[59]	Primary Biliary Cirrhosis	7	33-58	IV	$0.5x10^6$/kg	3 (monthly)	12	none
Zhang, 2013[60]	HIV	7	26-49	IV	$0.5x10^6$/kg	3 (monthly)	12	none
Jin, 2013[61]	Spinocerebellar Ataxia	16		IV & IT	N/A	N/A	12	none
Wu, 2013[62]	Leukemia, undergoing CBT	8	3-12	IV	$2\text{-}10x10^6$/kg	1	8-27	none
Jiang, 2013[63]	Stroke	4	40-59	Intra-arterial (MCA)	$2x10^7$	1	6	none
Hu, 2013[64]	Type I Diabetes	15	17.6	IV	$1.5\text{-}3.2x10^7$	1	24	none
Shi, 2012[65]	Liver Failure	24	24-59	IV	$0.5x10^6$/kg	3 (monthly)	18	none
Zhang, 2012[66]	Liver Cirrhosis	30	25-64	IV	$0.5x10^6$/kg	3 (monthly)	12	none
Qu, 2009[67]	Bone Nonunion	36	36	Intrabone	$1x10^6$ - $1x10^7$	1	13	none
Totals: 45 studies		**819**	**0-68**			**>1,700**	**3-84**	**6 studies (3 IT route)**

Abbreviations: HA-headache, IM-intramuscular, IT-intrathecal, IV-intravenous, N/A-not available, NR- not recorded

Experience with MSCs in Children:
The most well-studied MSC product given to children to date is a bone marrow-derived product originally manufactured by Osiris as Prochymal® and subsequently acquired by Mesoblast as Remestemcel-L used to treat patients with GvHD after a hematopoietic stem cell transplant. Prochymal® received regulatory approval in Canada in 2012 and in Japan in 2018 as TEMCELL® for use in children with acute steroid refractory GvHD. A recent expanded access study of Remestemcel-L enrolled 241 children (median age 9.6 years) with refractory GvHD from 2007-2014.[68] Eight MSC doses ($2x10^6$ cells/kg/dose) were given intravenously over a four week time period, with four additional weekly infusions in patients who demonstrated a partial or mixed response. There were no incidences of ectopic tissue formation in 2434 total doses, with follow-up of 2-9 years. The most frequent severe adverse events were infections (24%) and respiratory disorders (16%), common issues in post-transplant patients. There were only 11 severe adverse events and one infusion reaction related to MSC treatment. Patients with primary steroid-refractory GvHD who had only been treated with steroids demonstrated an 81% response rate to MSCs. Overall, 65% of patients demonstrated an improvement in their GvHD, with higher response rates in patients with less severe disease. This demonstrates that repeated doses of MSCs are safe and well-tolerated in children, and suggests that they provide anti-inflammatory and immunomodulatory effects in the setting of GvHD. A more recent phase III open-label, single arm study of Remestemcel-L in 55 children with primary steroid refractory GVHD replicated these results.

In a recent study, a German group pooled bone marrow cells from 8 healthy donors to create an MSC cell bank.[69] They treated 26 children, ages 1-19 years old with steroid-refractory GvHD, with a total of 81 doses of MSCs from this bank (median 3 doses per patient, median $2.2x10^6$/kg MSCs IV per dose). Treatment was well-tolerated, with only one reported headache and one episode of nausea across all doses. Six patients died from causes unrelated to MSCs (thromboembolism, disease progression, GvHD, sepsis). Response rate, defined as complete or partial remission of GvHD, was 77%.

Others are investigating the use of MSCs in patients with other conditions. There have been case reports of intracoronary administration of autologous bone marrow-derived MSCs in children, ages 4 months to 14 years, with severe dilated cardiomyopathy and/or heart failure with subsequent short- and intermediate-term improvement in clinical condition and B-type natriuretic peptide values.[70-72] Studies have also been conducted in patients with type 1 diabetes.[38] One such study administered umbilical cord-derived MSCs intravenously in 29 patients, including several children, with newly diagnosed type 1 diabetes.[64] Treatments were well-tolerated, and moderate improvements in some metabolic measures were observed. Early phase studies of MSCs are also underway in preterm infants with bronchopulmonary dysplasia. In one published study, cells were delivered intratracheally and no significant side effects were reported in these young babies.[55] None of these early phase studies have reported toxicity related to the MSC therapy. Per Table 1, over 105 children with various conditions have been treated with human umbilical cord tissue-derived MSCs without apparent adverse events.

MSCs in Neurologic Conditions:
Numerous preclinical studies using MSCs for diseases of the central nervous system suggest that MSCs can act through release of different neurotrophic, anti-inflammatory, and anti-apoptotic factors to promote recovery the injured area and prevent further damage. The majority of clinical experience has focused on adults with stroke, with a few studies in patients with neurodegenerative conditions or multiple sclerosis (MS).

Several small studies of autologous bone marrow-derived MSCs have been conducted in adult patients with acute or chronic stroke, with no significant side effects.[73] In a phase II study of an allogeneic MSC product (MultiStem) conducted in 126 adult patients with stroke (65 treated, 61 placebo), MSC therapy was well-tolerated.[74] While there was no difference between placebo and treated patients in measures of stroke recovery, the treatment group had a lower rate of mortality and infections, associated with down regulation of inflammatory biomarkers including IL-6. Patients who received MSCs earlier (24-36 hours post-stroke vs. 36-48 hours post-stroke) demonstrated more favorable recovery than patients who received later treatment or placebo.

A few clinical trials of autologous MSC therapy have been conducted in patients with multiple sclerosis (MS). Of 25 patients with progressive MS treated with a single intrathecal injection of autologous bone marrow-derived MSCs, the disease stabilized in half of patients over a one-year time period.[75] Side effects, all transient and self-limited including low-grade fever, nausea/vomiting, lower limb weakness, and headache, were likely related to the intrathecal route of administration and were consistent with those reported from other small studies of intrathecal MSC treatment in patients with MS.[76,77] Another study that treated 10 patients with progressive visual deficits due to MS with a single intravenous dose of autologous bone marrow-derived MSCs demonstrated improvements in visual acuity, visual evoked response latency, and optic nerve area.[78]

One clinical trial has been conducted in seven patients with Parkinson's disease who received a single dose of autologous bone marrow-derived MSCs transplanted to the subventricular zone using stereotaxic surgery. With a follow-up of 10 to 36 months, three patients demonstrated an improvement in disease symptoms. Two patients also reported subjective improvement of symptoms and reduction in drug dosage.[79]

Potential Mechanism of MSCs in ASD:
The exact mechanism of action of MSCs in ASD is the subject of ongoing investigations, but there are several potential means through which MSCs may exert therapeutic effects, including cell-mediated immunomodulation, molecular-mediated neuroprotection, and restoration of functional neurologic circuitry.

MSCs have been shown to exert immunomodulatory effects on cell-mediated immune responses by inhibiting T-cell proliferation and decreasing production of pro-inflammatory cytokines, including TNF-α and interferon gamma.[80] As ongoing immune dysregulation may contribute to the pathophysiology of ASD, suppressing the cell-mediated immune response with MSCs could have potential therapeutic benefit. hCT-MSCs have demonstrated the ability to suppress T-cell response in vitro, as measured by thymidine uptake four days after incubation of mononuclear cells with increasing numbers of hCT-MSCs and CD3/CD28 beads to stimulate T-cell proliferation (figure 2).

MSCs may also provide neuroprotection through anti-inflammatory mechanisms by inhibiting neural apoptosis, microglial activation, astrocyte proliferation, and oxidative stress molecules.[81] Koh, et al.[82] demonstrated that cord tissue-derived MSCs promote neuron survival via secretion of neurotrophic factors. In several other models, MSCs have demonstrated the ability to decrease both the number and activation of microglial cells, which are thought to play a critical role in the development of ASD.[83,84] It is unclear if this phenomenon is caused by a direct effect of the MSCs or if it is mediated through activation of cytokines (ie. TCP, IL-6).

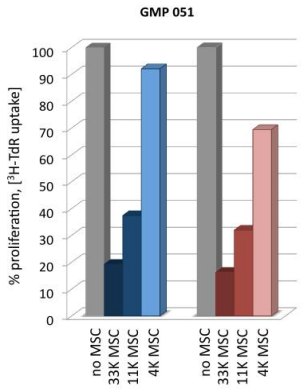
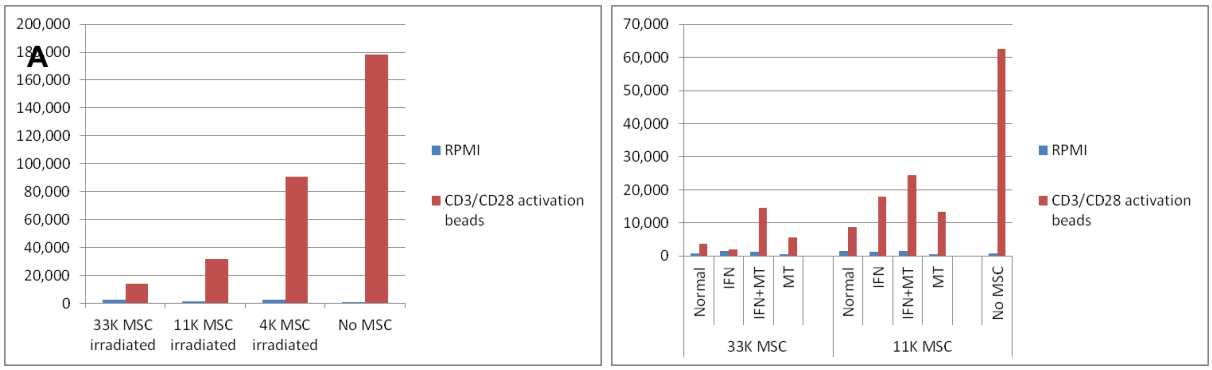


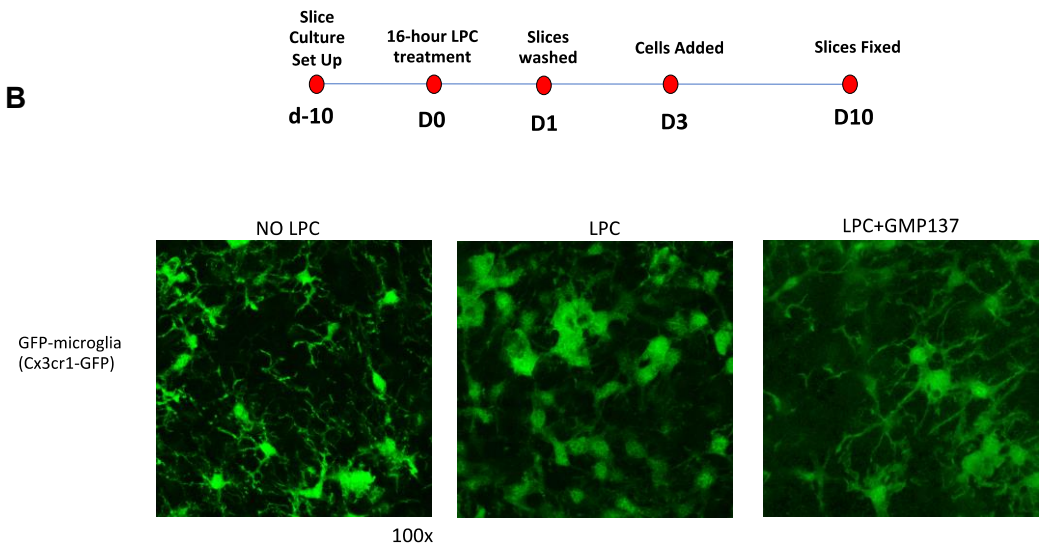
Figure 2. hCT-MSCs suppress T cell proliferation
MSC were thawed and plated in fibronectin-coated wells of a 96 well plate. After allowing the MSC to adhere overnight, T cell proliferation assays were established either in the presence or absence of the MSC. T cell proliferation assays were performed in the presence of a diminishing number of MSC, with the highest being 33,000 MSC per reaction. In each assay, 100,000 MNC were incubated with anti-CD3/CD28 beads to stimulate T cell proliferation. After 4 days, the level of cell proliferation was measured, using ^{3}H-thymidine incorporation. The data represent the responses of two different blood donors when cultured with one of the cell lines produced during validation runs.

Potency: Inhibition of T-cell Proliferation



No Interferon (IFN)

With Interferon (IFN) and/or MT



Finally, MSCs may also aid in synaptogenesis and restoration/regeneration of functional neurological pathways by supplying bioactive agents that stimulate the action of intrinsic neural progenitor cells. Various molecular targets have been implicated, including tissue plasminogen activator (tPA), synaptophysin, brain-derived neurotrophic factor (BDNF), and neurotransmitter receptors. Through a series of *in vitro* experiments via coculture, patch-clamp, inhibitory, and biochemical techniques, Koh, et al.[82] demonstrated that (1) human umbilical cord-tissue derived MSCs can induce synapse formation and enhance synaptic function and (2) thrombospondin proteins are both produced by cord tissue-derived MSCs and necessary for their synaptic effects. In addition to chemokines associated with the capacity to repair endothelium, hCT-MSCs also produce thrombospondin-1, indicating that they may also have the capacity to restore effective synapses. Research at Duke University demonstrated that hCT-MSCs produce and secrete multiple cytokine and chemokines using cytokine arrays by RayBiotech (Norcross, GA). Follow up studies using Bioplex assays and ELISA measured levels of selected cytokines/chemokines as shown in Table 2.

Table 2. Cytokine and chemokine production by hCT-MSCs.

Cytokine/chemokine	P2 supernatant range, pg/mL
BioPlex	
IL-6	178 - 1134
CCL2 /MCP-1	270 - 453
CXCL1/GRO	250 - 1280
CXCL5	880 - 3025
CXCL8/IL-8	250 - 837
ELISA	
Thrombospondin-1	150 - 415
A Bioplex has validated many of the findings from the cytokine arrays. ELISA assays have been used to quantify the production of Thrombospondin-1 and -2.	

2.5 PRECLINICAL STUDIES OF CELL THERAPIES IN ASD

Mouse models of single gene disorders that are associated with the ASD phenotype, such as Fragile X syndrome, Rett syndrome, and Tuberous Sclerosis Complex syndrome, have been used to study the effects of cellular therapy, such as MSCs, on both brain and behavior. Reported data from such animal studies may reflect differences related to the specific genetic subtype of ASD and may not generalize to all cases (e.g. idiopathic ASD).

Derecki et al[85] report recovery of function in a mouse model of Rett syndrome, an X-linked condition associated with ASD typically caused by a mutation of the *MECP2* gene. This gene encodes a methyl-CpG-binding protein, and the mutation leads to deficient phagocytic function in glial cells. Transplantation of cells from wild type bone marrow via intravenous infusion arrested disease development in the mouse model of Rett syndrome (*Mecp2*-null C57BL/6 mice). Following engraftment, survival was improved, breathing patterns normalized, apneas were reduced, body weight increased, and locomotor activity was improved.

The BTBR *T*$^{+}$ *Itpr3*^{tf}*/J* (BTBR) mouse strain, derived from the inbred Black and Tan BRachyury strain, is another mouse model of ASD. In addition to impaired social behavior, aberrant communication, increased repetitive behaviors, and increased cognitive rigidity, BTBR mice also exhibit increased levels of peripheral CD4+ T-cells, peripheral B-cells, and serum and brain

immunoglobulin levels, among other immune abnormalities.[86] Segal-Gavish, et al[87] delivered human MSCs to BTBR mice via intraventricular injection into the central nervous system. Mice were immunosuppressed with cyclosporine before and after treatment. In this model, improvements in all three domains – social behavior, stereotyped behaviors, and cognitive rigidity – were observed in MSC-treated mice compared to controls. Differences in anxiety-related behaviors and locomotion were not observed.

These mouse models demonstrate the potential for benefit from cellular therapies in at least certain subtypes of ASD.

2.6 OUTCOMES OF CELL THERAPIES IN CHILDREN WITH ASD

The autism and cellular therapies teams at Duke have partnered to test whether intravenous infusions of autologous or allogeneic cord blood or allogeneic cord tissue derived MSCs would be safe, feasible and improve core symptoms of autism in affected individuals. Results of these studies are summarized below.

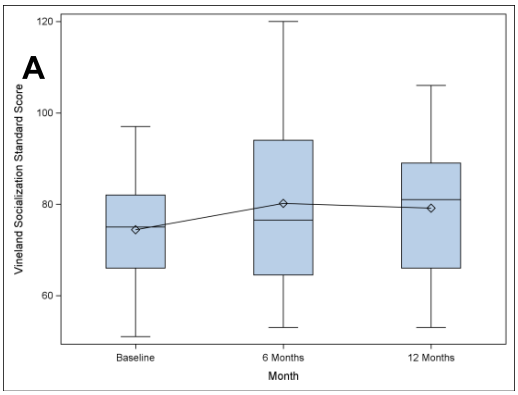
DukeABCs Clinical Trial: Duke investigators recently completed and published the results of an open-label Phase 1 safety and tolerability study in 25 children (ages 2-5 mean age 4.5 years), diagnosed with ASD who were treated with a single intravenous infusion of ***autologous*** CB and followed for a year (ClinicalTrials.gov ID: NCT02176317).[88] CB was administered as a single infusion (median infused dose: $2.6x10^7$/kg, range: $1.0x10^7$/kg to $8.1x10^7$/kg). No immunosuppression was administered prior to infusion.

The safety and tolerability profile of autologous CB infusion in ASD was excellent. No serious adverse events were reported, and adverse events, in general, were sparse. Three children had mild allergic reactions associated temporally with the infusions, consisting of cough and hives during infusion for 1 child and cough post-infusion for 2 children. Also, one child was noted to be more irritable for 2 days post-infusion. No participants discontinued prematurely from the study due to adverse events.

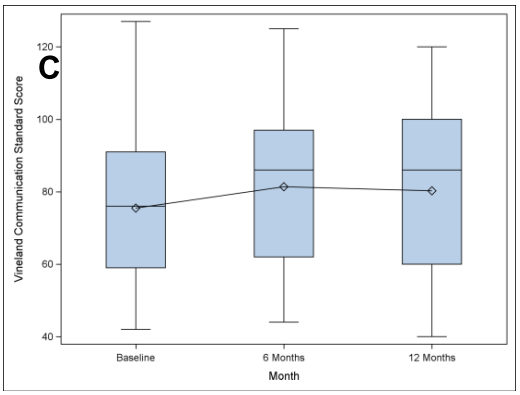
With regard to preliminary assessments of efficacy, improvements in social communication abilities were noted on the caregiver-completed Vineland Adaptive Behavior Scales-Second Edition (VABS-II) and on the Pervasive Developmental Disorder Behavior Inventory (PDDBI) (figure 4). The Clinical Global Impression-Improvement scale, completed by clinicians, reflected beneficial changes during the 6-month period post-infusion in core ASD symptoms in approximately 60% of the participants, as manifested by the participants' increased social communication skills, receptive/expressive language, decreased repetitive behavior, and decreased sensory sensitivities. In computerized eye-tracking assessments, the participants manifested improvements in social attention. Participants, on average, showed a 20% increase in odds of gazing at the actress' eyes over time (OR=1.20, 95% CI: 1.00, 1.43, p5.048). A 7-point change in VABS-II socialization standard score was associated with a 14% increase in odds of gazing at the actress (OR=1.14, 95% CI: 1.07,1.21; p<.001).[88]

Electrophysiological recordings were taken during viewing of dynamic social and nonsocial stimuli at 6 and 12 months post-treatment. Significant changes in EEG spectral characteristics were found by 12 months post-infusion, which were characterized by normalization of the EEG spectrum, reflected in increased alpha and beta power and decreased EEG theta power.[89]

Figure 4: Changes in ASD Symptoms after Autologous CB Infusion. Panel A: Vineland-II socialization domain standard scores at baseline and six months after infusion (p=0.02 baseline to six months). Panel B: Distribution of Vineland-II socialization domain standard scores stratified by nonverbal IQ. Panel C: Vineland-II communication domain standard scores at baseline and six months after infusion (p<0.01 baseline to six months). Panel D: Distribution of CGI-I scores at six (blue) and 12 (red) months post-infusion. Sample sizes are N=25 for baseline and 6-month time points and N=22 at 12-months.

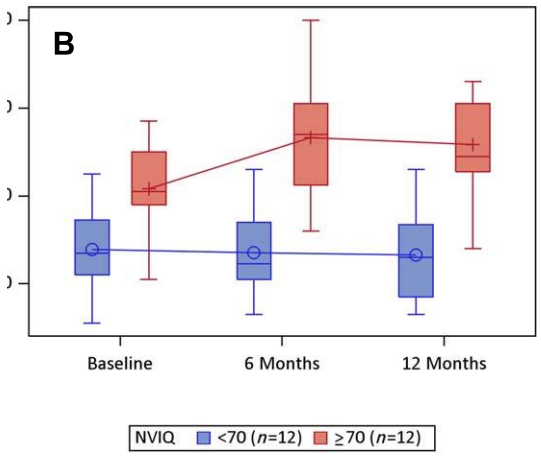


Time Point	Median	Mean	SD
Baseline	75.00	74.40	11.41
6 Months	76.50	80.17	17.88
12 Months	81.00	79.13	16.68

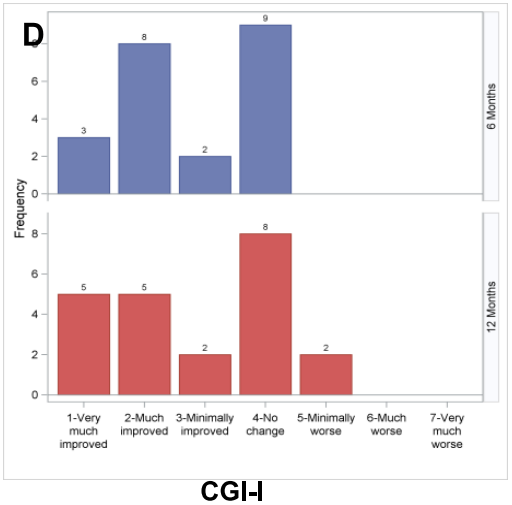


Time Point	Median	Mean	SD
Baseline	76.00	75.48	20.32
6 Months	86.00	81.38	22.75
12 Months	86.00	80.25	23.34

Vineland Communication Domain



Vineland Socialization Domain



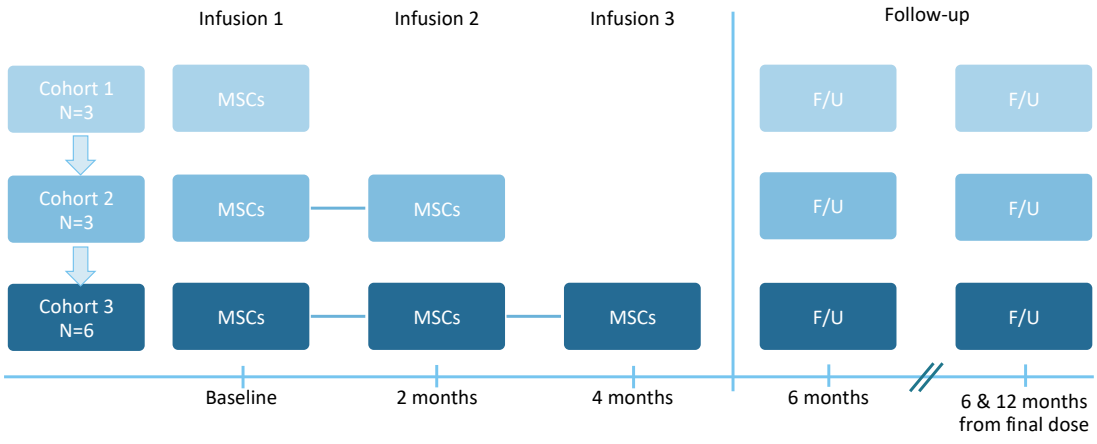
CGI-I

There was also some evidence that improvements in social behavior were found to be associated with increases in connectivity across brain regions when assessed via diffusion tensor imaging (obtained via MRI scans). In an open label trial evaluating the efficacy of autologous cord blood for treating autism symptoms, improvement on 3 behavioral outcome measures was correlated with increased connectivity between the left temporal pole and the left hippocampus (VABS II $p<0.001$; Expressive One Word Picture Vocabulary Test-Fourth Edition (EOWPVT-4) $p<0.05$; CGI-I $p<0.0001$). Improvement on both the VABS-II and the EOWPVT-4 was correlated with increased connectivity between the fusiform and putamen (VABS II $p<0.05$; EOWPVT-4 $p=0.01$) as well as between the inferior temporal gyrus and the hippocampus (both $p<0.05$), both in the left hemisphere. Finally, there was a significant correlation between improvement on both the VABS-II and the CGI-I and increased connectivity between the inferior temporal gyrus and superior temporal gyrus (VABS-II $p<0.05$, CGI-I $p<0.01$) and between the temporal pole and globus pallidus (VABS II $p<0.01$; CGI-I $p<0.05$) in the left hemisphere. Improvement on the VABS II and CGI-I was also significantly correlated with increased connectivity between the frontal pole and globus pallidus and the insula and putamen in the right hemisphere (all $p<0.05$). Based on these data, a Phase 2 randomized study was conducted to evaluate the efficacy of CB therapy versus placebo in children with ASD. Data analysis of this trial is currently in progress.

MSCs in ASD: One Chinese study enrolled 37 children with ASD and treated them with 4 doses of either umbilical cord blood mononuclear cells (CBMNC, given both intravenously and intrathecally) (n=14), CBMNC + intrathecal umbilical cord-derived mesenchymal stem cells (UCMSC) (n=9), or standard therapy (n=14).[34] The only treatment-related side effect was transient fever in five patients. At six months post-treatment, both treated groups demonstrated greater improvement in multiple ASD measures (Childhood Autism Rating Scale, Clinical Global Impression scale, Aberrant Behavior Checklist) than the placebo group, indicating a potential therapeutic response.

Results of a Phase I Trial of human cord tissue MSCs in children with ASD: At Duke, we have treated 12 children, ages 2-11 years, with ASD with hCT-MSC on a phase I open label trial to determine the safety of single and repeated IV doses of hCT-MSC. In this study, the first cohort of three patients received a single dose, the second cohort of three patients received two doses given two months apart, and the third cohort of six patients received three hCT-MSC infusions with a two-month interval between doses. Each dose contained $2x10^6$/kg hCT-MSC (see study schema in Figure 5).

Figure 5: Study Design for Phase I Study of hCT-MSC in Children with ASD



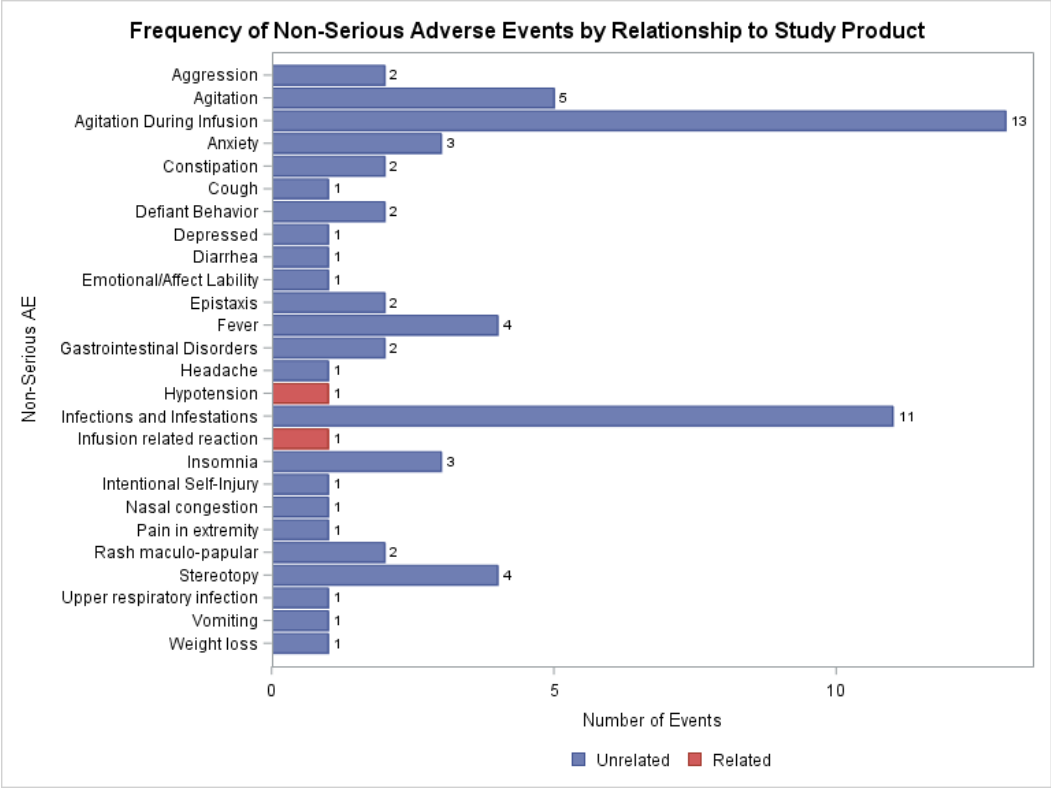
Twelve children, three girls, nine boys with a mean age of 6.4 years (range 4-9 years), were enrolled between June and October 2017. Eleven participants were white, one was Asian; two were Hispanic or Latino. A total of 27 hCT-MSC doses were administered from 3 different manufacturing lots. Target dose at each administration was $2.0x10^6$ TNC/kg based on the post thaw count. The mean of the actual dose infused was $2.0x10^6$ TNC/kg with a standard deviation of $0.4x10^6$ TNC/kg. One patient received a lower dose of $1.79x10^6$ TNC/kg (Patient 12 at Infusion 3) due to a laboratory complication. All children were premedicated with an intravenous dose of diphenhydramine 0.5mg/kg and solumedrol 0.5mg/kg approximately 30 minutes before the MSC dose. The MSCs were administered over 30 minutes. IV hydration was administered for 15-60 minutes post MSC infusion.

Three reactions were reported in 2 patients within 24 hours of infusion. One patient in Cohort 2 experienced Allergy and Moderate Hypotension during infusion 2 associated with a deviation in the premedication schedule for the infusion. Due to an adverse reaction of extreme agitation to IV Benadryl during the initial infusion, this patient received oral Atarax as an alternative premedication for the second infusion. Shortly after initiation of the second hCT-MSC infusion, the patient developed diffuse erythroderma and a cough followed by mild hypotension and hypoxia. The infusion was immediately stopped, and the patient recovered completely with a fluid bolus and an extra dose of IV Solumedrol. The remainder of the hCT-MSC infusion was successfully completed after a dose of IV Benadryl. One patient in Cohort 3 experienced Moderate Hypotension after infusion 3 and received additional IV fluids.

A total of 66 non-serious Adverse Events (AEs) that were not specifically attributed to the study product were reported among 11 of the 12 enrolled participants (Figure 6). All of these reported events were Mild. The most frequently occurring event, agitation during the infusion procedure, was associated with the requirements of placing and maintaining an IV and being confined to a hospital room for the infusion, and all of these events resolved on the same day. A total of 22 other psychiatric or behavioral symptoms were reported in 7 different participants: 2 participants/2 events in Cohort 1, 1 participant/5 events in Cohort 2, and 4 participants/15 events in Cohort 3. These symptoms included aggression (n=2), agitation (n=5), anxiety (n=3), defiant behavior (n=2), depression (n=1), emotional labiality (n=1), insomnia (n=3), intentional self-injury (n=1), and stereotopies (n=4). Of note, 3 participants accounted for 17/22 of the psychiatric or behavioral adverse events. Aside from agitation during the infusion procedure, there was a trend of increasing frequency of non-serious adverse events with increasing number of doses administered, but this was not statistically significant. There were no differences observed in the frequency of AEs according to lot number (GMP-075: median=9 events/participant, range: 1-12; GMP-087: median=4.5, range: 0-13; GMP-088: median=3.5, range: 3-50) ($P_{Kruskal\text{-}Wallis}$=0.58). There were no concerning changes in blood counts, chemistries, basic inflammatory markers (CRP, ESR), or humoral and cellular immune profiles throughout the study. All participants remained Coombs negative.

Six months after the initial hCT-MSC dose, development of anti-HLA class I antibodies was observed in 5 of 9 participants who did not have detectable HLA antibodies pre-treatment. HLA antibody data is available on all 12 participants at baseline and 6 months, and 11/12 participants at ≥12 months after their final hCT-MSC dose. In those participants who developed new HLA antibodies by six months, the HLA antibodies persisted at ≥12 months (figure 7). The HLA antibodies were directed against HLA alleles/antigens expressed on the MSCs and not by the patient. There were no associated clinical events in patients who developed anti-HLA antibodies.

Figure 6: Adverse Events Observed during Phase I hCT-MSC Study. A total of 2 events were related to study product—Hypotension and Infusion Related Reaction (discussed above).



Class I HLA antibodies by number of doses, lot of hCT-MSC, and degree of HLA match are shown in Figure 5. New class I anti-HLA antibodies developed in 1/3 participants who received one dose of hCT-MSC, 1/3 participants who received 2 doses, and 3/6 participants who received 3 doses. All three participants who developed broad-spectrum class I HLA antibodies were treated with the same lot of hCT-MSC. In addition, 4/4 participants who were at least haploidentical to their hCT-MSC donor developed new-onset HLA antibodies (broad spectrum or donor-specific), versus 1/8 participants who were 0/8, 1/8, 2/8, or 3/8 HLA-matched at HLA-A, B, C, and DRB1. No detected anti- HLA antibodies have been clinically significant. With the small numbers of patients enrolled on this study, there was no ability to assess effect of numbers of doses administered on the development of these antibodies.

Results of behavioral outcome assessments are shown in Table 3. Measures reported below include assessments of social communications skills (VABS-3) with increases of 3 points and above indicating improvement, severity of autism symptoms (PDDBI) with decreases of at least 5 points indicating improvement, and expert clinical judgment (CGI) ranging from no improvement to much improvement. Fifty percent (6/12) of patients showed an improvement on at least 2/3 measures, 33% (4/12) showed an improvement on all three measures, and 16% (2/12) showed an improvement on 2/3 measures. Of the children who improved on 2/3 of the outcome measures, two had one dose, two had two doses, and two had three doses.

Figure 7: 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.

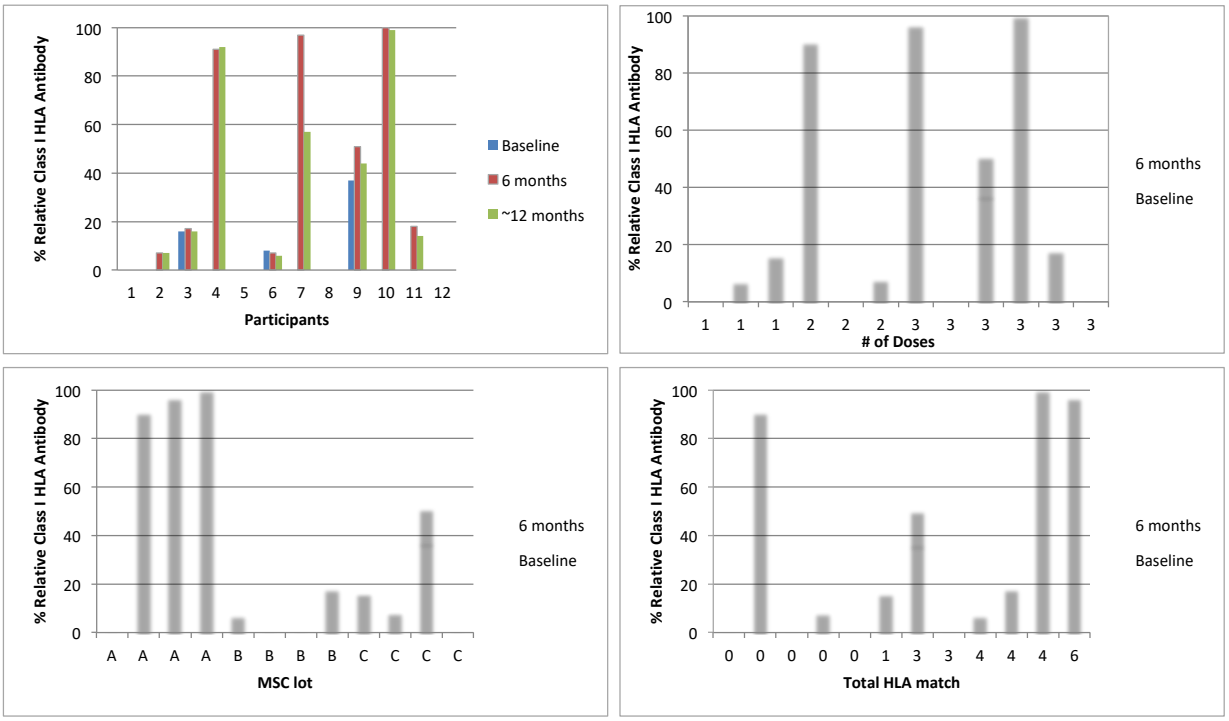


Table 3: Behavioral Evaluations, Phase I Study of hCT-MSC in Children with ASD

ID	Dose	Sex	Nonverbal DQ	VABS*	PDDBI	CGI	# of assessments indicating 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	Min	1
8	3	M	54	-8	-4	No	0
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	No	1

DQ=Developmental Quotient, VABS=Vineland Adaptive Behavior Scales-Third Edition (VABS-3) Socialization Standard Score, PDDBI=Pervasive Developmental Disorder Behavior Inventory Autism Composite, CGI=Clinical Global Impression-Improvement scale
*Clinically significant improvement = 3 points.

2.7 STUDY RATIONALE AND HYPOTHESIS

The mechanistic rationale and overarching theory of this line of investigation is that hCT-MSC can act through paracrine and trophic mechanisms to favorably modulate ongoing inflammation and/or immune pathology in the brain, suppress microglial activation, and possibly protect neurons from further damage. The primary hypothesis of this phase I clinical trial is that administration of hCT-MSCs will be safe and tolerable and result in improvement in the social communication abilities, as measured via objective ratings of social communication abilities during caregiver-child interaction.

In many contexts, MSCs suppress, rather than augment, immunological and inflammatory responses. Documented mechanisms include shifts in effector T cells such as generation of regulatory T cell populations and changes in monocyte/dendritic cell cytokine generation leading to anti-inflammatory cytokines. Therefore, it is plausible to consider a population of MSCs as an immunological and/or anti-inflammatory agent. Both postmortem brain tissue studies and PET imaging data from living individuals with ASD have revealed evidence of increased microglial activation, suggesting that immune and/or inflammatory mediated brain damage plays a role in the etiology of ASD as discussed above.[90] Thus, hCT-MSC may be a candidate therapy for ASD because of the immunomodulatory activities of MSCs.

2.8 STUDY DESIGN

This is a single site, prospective study of one intravenous infusion of human umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC) in toddlers with autism spectrum disorder (ASD). Toddlers 18 months to <4 years (3 years 364 days) of age with a confirmed diagnosis of ASD will be eligible to participate. Diagnosis will be confirmed at the time of the eligibility visit at the Duke Center for Autism and Brain Development. All participants will receive a single intravenous dose of $2x10^6$/kg hCT-MSC per kilogram at baseline. Assessments will be conducted at baseline and 6 months, with remote follow-up assessments at 12 months.

The primary purpose of this study is to evaluate safety and feasibility. Safety assessments include monitoring of acute infusion reactions, adverse events, incidence of infections, and markers of alloimmunization. Clinical outcome measures will also be described. A key clinical outcome measure is the change in social communication abilities from baseline to 6 months based on the Joint Engagement Rating Inventory (JERI), a commonly-used and well-validated coding system for rating the quality and quantity of social communication skills in toddlers with and without ASD.[91] JERI coding rates social communication abilities on a 1 to 7 scale and factors in both the quantity and quality of skills. The total joint engagement score as well as ratings on all JERI subscales that comprise the total score will be described.

Other clinical endpoints will include the PDD Behavior Inventory (PDDBI) autism composite score, the mean of the Socialization Subscale Standard Score and Communication Subscale Standard Score on the Vineland Adaptive Behavior Scales (VABS-3), the Clinical Global Impression Scale (CGI) – Severity and Improvement Scales, the Communicative Development Inventories (CDI-2): Words & Sentences subscales, and the Pediatric Quality of Life Scale (PedsQL Generic Core Scale).

Exploratory clinical endpoints will include autism symptoms measured by an app that elicits and records autism symptoms on an iPad (SenseToKnow), PDD Behavior Inventory (PDDBI) Subscales, and VABS-3 Standard Score and age equivalent for the following subscales: Socialization, Communication, and Daily Living and the Standard Score and age equivalent for the VABS-3 Adaptive Behavior Composite.

Safety and VABS-3 assessments will also be conducted remotely at three and 12 months. Duration of study participation will be 12 months from the time of the baseline infusion.

2.9 RISKS AND BENEFITS

Potential risks associated with infusion of MSCs include a reaction to the product (rash, shortness of breath, coughing, wheezing, difficulty breathing, hypotension, hypertension, hypoxia, swelling around the mouth, throat or eyes, tachycardia, bradycardia, diaphoresis), transmission of infection, and HLA sensitization. Theoretical risks that must be considered but have not been associated with MSC administration in humans include the possibility of immune suppression and ectopic tissue formation. Cord blood collected with the cord tissue used to manufacture hCT-MSC is screened for infection, and the product must meet release criteria prior to infusion, as described below. Participants will not receive immunosuppressive therapy prior to or after infusion of hCT-MSC cells. Additionally, an inherent risk in completing study procedures in person is possible exposure to COVID-19; however, social distancing, screening, masking, cleaning and other safety procedures in line with the most recent guidelines from Duke Health and the Duke University School of Medicine have been implemented to minimize this risk as much as is feasible.

Potential benefits of this intervention include the possibility that hCT-MSC may, via direct or indirect mechanisms, induce changes that result in the reduction of the participant's core ASD symptomatology and improvement in abilities affected by ASD symptoms.

3.0 STUDY OBJECTIVES

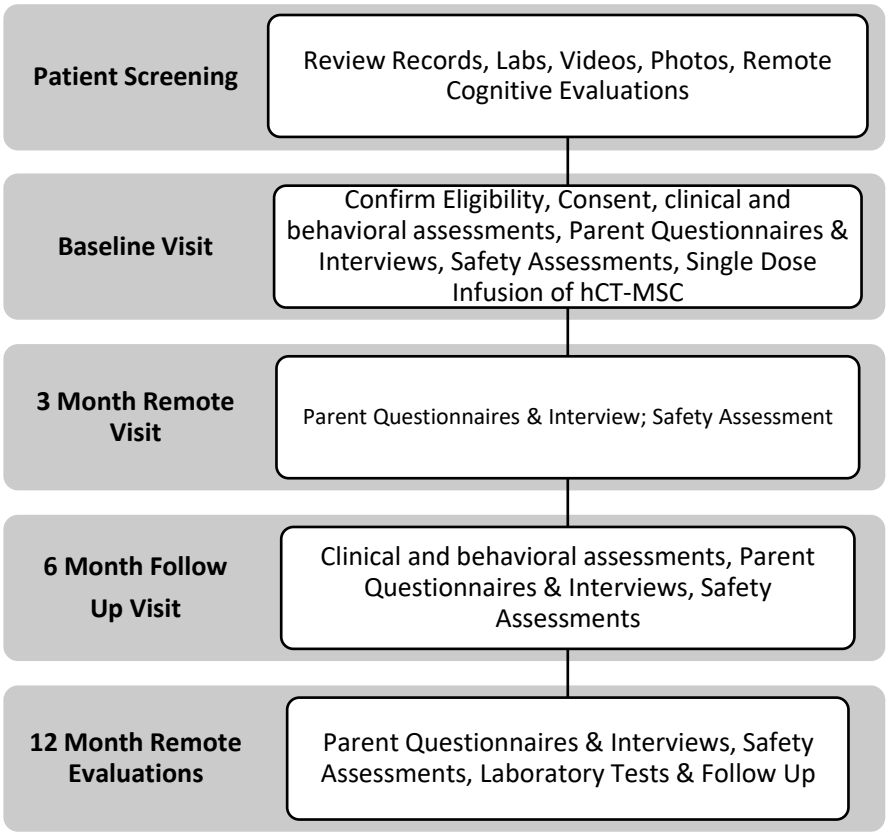
1. To determine the safety and tolerability of a single intravenous infusion of hCT-MSC in very young children with ASD.
2. To determine whether there are improvements in social communication abilities during caregiver-child interaction from baseline to six months following an infusion at baseline.

4.0 STUDY DESIGN

4.1 GENERAL DESIGN

This is a single site, phase I, open-label, prospective study of one intravenous infusion of human umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC) in 12 toddlers ≥18 months to <4 years (3 years 364 days) of age with autism spectrum disorder (ASD). All participants will receive a single intravenous dose of $2x10^6$ hCT-MSC per kilogram at baseline. The primary outcome is safety, as measured by the incidences of infusion reactions, infections, and new-onset clinically significant anti-HLA antibodies. The main clinical outcome measure is the change in social communication abilities from baseline to 6 months based on the Joint Engagement Rating Inventory (JERI).[91] Duration of study will be 12 months from the time of the initial infusion.

4.2 STUDY FLOW CHART



4.3 STUDY ENDPOINTS

Safety Endpoints:
Safety of hCT-MSC infusion in children with ASD will be assessed by:

1. Incidence and severity of infusion reactions
2. Incidence and severity of product-related infections
3. Evidence of alloimmunization via anti-HLA antibodies
4. Incidence and severity of graft vs. host disease
5. Incidence and severity of unexpected adverse events, by relation to study product

Efficacy Endpoints:
The primary clinical outcome measure is the change in social communication abilities from baseline to 6 months based on total score (1-7) on the Joint Engagement Rating Inventory (JERI), a commonly-used and well-validated coding system for rating the quality and quantity of social communication skills in toddlers with and without ASD.[91]

Change measured on the following endpoints from baseline to six months will be compared:

1. PDD Behavior Inventory Autism Composite Score (PDDBI)
2. Mean of the Socialization Subscale and Communication Subscale standard scores on the Vineland Adaptive Behavior Scales (VABS-3)
3. Clinical Global Impression Scale (CGI) – Severity and Improvement (Improvement at 6 months only

4. Communicative Development Inventories (CDI-2): Words & Sentences subscales

Exploratory Endpoints*:*
Change measured from baseline to six months will be evaluated on the following outcome measures.

1. Changes in auditory orienting, facial expressions, look duration, attention to social and nonsocial stimuli, postural sway, and vocalizations as measured via computer vision analysis by an app that elicits and records autism symptoms on an iPad (SenseToKnow)

2. PDD Behavior Inventory (PDDBI) Subscales
3. VABS-3 Socialization Subscale Standard Score and age equivalent
4. VABS-3 Communication Subscale Standard Score and age equivalent
5. VABS-3 Daily Living Subscale Standard Score and age equivalent
6. VABS-3 Adaptive Behavior Composite Standard Score and age equivalent
7. Pediatric Quality of Life Scale – Generic Core

5.0 RESEARCH PARTICIPANT SELECTION AND WITHDRAWAL

5.1 STUDY POPULATION

Twelve toddlers ages 18 months to <4 years (3 years 364 days) with a confirmed diagnosis of ASD.

5.2 INCLUSION CRITERIA

1. Age 18 months to <4 years (3 years 364 days) at the time of consent
2. Confirmed clinical DSM-5 diagnosis of Autism Spectrum Disorder using the DSM-5 Checklist as informed by the Brief Observation of Symptoms of Autism (BOSA) Assessment.
3. Fragile X testing performed and negative; constitutional microarray (CMA) and/or whole exome sequencing performed and results not linked to autism diagnosis
4. Stable on current psychoactive medication regimen (dose and dosing schedule) for at least 2 months prior to infusion of study product
5. Normal absolute lymphocyte count (≥1500/uL)
6. Participant and parent/guardian are English speaking
7. Able to travel to Duke University for two multi-day visits (baseline and six months) and parent/guardian is able to participate in interim surveys and interviews
8. Parental consent from at least one parent or guardian.

5.3 EXCLUSION CRITERIA

1. General:
    a. Review of medical records indicates ASD diagnosis not likely
    b. Screening data suggests that participant would not be able to comply with the requirements of the study procedures as assessed by the study team
    c. Family is unwilling or unable to commit to participation in all study-related assessments, including protocol follow up
    d. Sibling is enrolled in this (Duke TACT) study
2. Genetic:
    a. Records indicate that child has a known genetic syndrome such as (but not limited to) Fragile X syndrome, neurofibromatosis, Rett syndrome, tuberous sclerosis, PTEN mutation, cystic fibrosis, muscular dystrophy or a genetic mutation known to be associated with ASD

   b. Known pathogenic mutation or copy number variation (CNV) associated with ASD (e.g., 16p11.2, 15q13.2, 2q13.3)
3. Infectious:
   a. Known active CNS infection
   b. Evidence of uncontrolled infection based on records or clinical assessment
   c. Known HIV positivity
   d. Exposure to COVID-19 in the preceding 14 days or positive COVID-19 test in the previous 28 days. Subjects with a past history of infection with COVID-19 must be symptom-free for 14 days prior to the initial visit

4. Medical:
   a. Known metabolic disorder
   b. Known mitochondrial dysfunction
   c. History of unstable epilepsy or uncontrolled seizure disorder, infantile spasms, Lennox Gastaut syndrome, Dravet syndrome, or other similar chronic seizure disorder
   d. Active malignancy or prior malignancy that was treated with chemotherapy
   e. History of a primary immunodeficiency disorder
   f. History of autoimmune cytopenias (i.e., ITP, AIHA)
   g. Coexisting medical condition that would place the child at increased risk for complications of study procedures
   h. Concurrent genetic or acquired disease or comorbidity(ies) that could require a future stem cell transplant
   i. Significant sensory (e.g., blindness, deafness, uncorrected hearing impairment) or motor (e.g., cerebral palsy) impairment
   j. Impaired renal or liver function as determined by serum creatinine >1.5mg/dL or total bilirubin >1.3mg/dL, except in patients with known Gilbert's disease
   k. Significant hematologic abnormalities defined as: Hemoglobin <10.0 g/dL, WBC < 3,000 cells/mL, ALC <1000/uL, Platelets <150 x 10e9/uL
   l. Known clinically relevant physical dysmorphology associated with neurodevelopmental conditions.
5. Current/Prior Therapy:
   a. History of prior cell therapy
   b. Current or prior use of IVIG or other anti-inflammatory medications with the exception of NSAIDs
   c. Current or prior immunosuppressive therapy
      i. No systemic steroid therapy that has lasted >5 days within 4 weeks prior to enrollment. Topical and inhaled steroids are permitted.

5.4 RESEARCH PARTICIPANT RECRUITMENT AND SCREENING

Patients may be recruited through IRB-approved advertising for the study on the websites of private CB banks, parent sponsored websites, the NMDP website, selected autism societies, local medical providers, or the Duke Center for Autism and Brain Development, through a record of inquiries for previous studies (brain injury database), and through the Duke Center for Autism and Brain Development research registry. Separate IRB approval will be obtained for any advertisements.

If a participant has consented to the Duke Center for Autism Research Repository (Pro00054178), study team members may access these data for purposes of screening, eligibility, or as source data in this study. Participants screened for or enrolled in the TACT study may also be approached per repository protocol to determine their interest in storing data from this study and

any future studies into the repository for future use. The consent form and participant-facing materials related to the Duke Center for Autism Research Repository are submitted and approved separately, through Pro00054178.

Screening for the study is conducted under a separate, IRB-approved screening protocol (Pro00063563). Under this protocol, after written informed consent is obtained from a parent/guardian, the patient's medical and psychological records, therapy records, photographs, behavioral videos, and results of genetic testing are obtained and reviewed by two teams. The medical review is conducted by a team of pediatric nurses, nurse practitioners, and physicians to identify the presence of any metabolic, immunologic, neurologic, sensory, genetic, or laboratory exclusion criteria. If no exclusion criteria are identified, the psychiatric review is then conducted by a combination of psychologists, psychiatrists, and other clinically trained staff with expertise in diagnosing and treating children with ASD. They perform an extensive review of the patient's psychological records as well as any therapy records available. A patient must be approved by both medical and psychiatric screening teams to proceed with further laboratory, phone, teleconference, or in-person screening and study enrollment. Should a concern for a previously undiagnosed condition or genetic finding arise during the screening process, this will be discussed with the patient's parent(s)/guardian(s) and a referral will be made to an appropriate medical or psychiatric provider for evaluation and treatment, if indicated.

5.5 EARLY WITHDRAWAL OF RESEARCH PARTICIPANTS

Criteria for Removal from Protocol Therapy:

1. Diagnosis of a genetic disease while under evaluation or on study.
2. Change in medical condition that precludes study participation.

Patients who are off protocol therapy are to be followed until they meet any one of the off-study criteria (see below). Follow-up data will be obtained on off-protocol participants unless consent is withdrawn. Participants that are taken off study prior to infusion of hCT-MSC will be considered not evaluable and can be replaced with another participant.

Off-Study Criteria:

1. Death.
2. Lost to follow-up.
3. Withdrawal of consent for any further data collection.
4. Completion of the final study visit.

6.0 STUDY PRODUCT

6.1 HUMAN UMBILICAL CORD TISSUE-DERIVED MESENCHYMAL STROMAL CELLS (hCT-MSC)

hCT-MSCs are a product of allogeneic cells manufactured from digested umbilical cord tissue that is expanded in culture, cryopreserved and banked. hCT-MSCs are manufactured from umbilical cord tissue donated to the Carolinas Cord Blood Bank, an FDA-licensed, FACT-accredited, public cord blood bank at Duke University Medical Center, after written informed consent from the baby's mother. Cord tissue is harvested from the placentas of male babies delivered by elective C-section after a normal, full-term pregnancy. Donor screening questionnaires are completed by the maternal donor, and maternal blood is tested for communicable diseases by the CLIA-certified donor screening laboratory. Donors must be eligible for donation to a public cord blood bank for allogeneic use. After delivery of the placenta and cord, the cord blood is aseptically drained from the placenta. Then the cord is dried and cleaned with chloropreps, separated from the base of the

placenta, placed in a sterile bottle containing Plasmalyte A, and transported to the Robertson CT2 GMP cell processing laboratory at room temperature in a validated container.

In the clean room manufacturing suite, in a biosafety cabinet, the cord tissue is removed from the media, placed in sterile dishes, cut into small pieces and then minced and digested in the Miltenyi Biotec GentleMacs Octo Dissociator with GMP-grade enzymes: hyaluronidase, DNase, collagenase, papain. The resultant cell suspension is placed in culture in Prime XV MSC Expansion XSFM (Irvine Scientific) media with 1% platelet lysate and grown to confluence (~7-14 days) to establish the P0 culture. To establish the master cell bank, P0 is harvested and cryopreserved in cryovials with Cryostor 10 media (BioLife), and stored in the vapor phase of liquid nitrogen. P1 and P2 cultures are grown under similar conditions, in hyperflasks or hyperstacks without platelet lysate, as needed to create the working cell bank and product for administration, respectively. Cells from P1 and P2 are removed from plastic cultureware using TrypLE (Gibco). The final product is derived from the P2 cultures which are harvested into plasmalyte with 5% human serum albumin, washed and cryopreserved in 5 compartment cryobags (Syngen) in 5 mL containing 50-100 million cells in a final concentration of 10% DMSO with dextran (Akron Scientific). On the day of administration, one compartment is thawed, diluted in 10-40 mls of plasmalyte IV solution, placed in a syringe or bag and transported to the bedside for administration over 30-60 minutes.

At each passage, the cell product is characterized by assessing cell surface phenotype by flow cytometry and functional assays via T-cell proliferation and organotypic models of microglial activation. Each lot, prior to cryopreservation of P2, will also be tested for sterility, endotoxin and mycoplasma and these tests must meet specifications. For dosing, release testing after thaw and dilution will include TNCC and viability via cellometer. Sterility cultures (14 day) are also initiated but results are not available at the time of infusion. See section 6.4 for management plan if sterility cultures subsequently turn positive post infusion. Patients will be dosed with $2x10^6$ hCT-MSCs/kg based on the post thaw count.

6.2 DONOR SCREENING AND TESTING

Donor screening and testing is performed per Carolinas Cord Blood Bank standard operating procedures to meet all requirements in 21CFR Part 1271. The screening and testing is current with recommendations and is approved by the FDA under biological license number 1870. Maternal donors of umbilical cord blood are screened and tested for HIV-1, HIV-2, hepatitis B virus (HBV, surface and core antigen), hepatitis C virus (HCV), Treponema pallidum (syphilis), CJD (screening only), Chagas, human T-lymphotropic virus types 1 and 2 (HTLV-1, HTLV-2) and CMV. Nucleic acid testing for HIV-1/2/O, HBV, WNV and HCV are also performed on maternal blood. Screening for Zika virus may also performed.

Because the cord tissue used for this study will be obtained from donors consented for cord blood donation to the Carolinas Cord Blood Bank, they will undergo donor screening and infectious disease testing per Carolinas Cord Blood Bank standard operating procedures. The cord blood-associated maternal samples and cord tissue MSC samples will be retained as reference samples for future testing as part of this study.

6.3 PROCESS AND FINAL FORMULATION

hCT-MSC is manufactured from a single umbilical cord tissue in a series of three steps that generate a master cell bank, a working cell bank, and the study product. The product for each step is frozen and stored in vapor phase in liquid nitrogen freezer. At P2, a representative cryobag

will be thawed and qualified prior to the treatment of any patients with that lot of product. Testing will include cell count, viability, phenotype, functional assays, endotoxin, mycoplasm, gram stain and sterility.

On the day of treatment, cells are thawed per SOP CT2-MSC-006, diluted in 10-40 mLs of plasmalyte-A + 5% HSA, and an aliquot removed for cell count, viability, and sterility culture. If the cells meet release criteria, the final product volume is adjusted to deliver the appropriate dose ($2x10^6$ cells/kg) to the study participant. The cells are delivered to the bedside in a bag or syringe containing plasmalyte-A, 5% HSA, and residual DMSO. Any removed cell suspension is inoculated into aerobic and anaerobic culture bottles for sterility testing. The cells have a four-hour expiry post thaw.

The hCT-MSC final product will be released conditionally for administration to the patient after testing a post thaw cell count and viability. Final release will occur after the 14-day sterility culture period for the study product. In the event that a sterility culture turns positive after administration of the product, the organism will be identified and antibiotic sensitivities performed. The patient's family will be contacted to determine if they are symptomatic (ie fever). Asymptomatic patients will not be treated with antibiotics. Symptomatic patients will be evaluated and treated accordingly, with blood cultures and antibiotics as appropriate. All patients receiving a product with subsequent positive sterility test will be followed with close, regular contact by a study nurse for 14 days after the positive sterility test is noted.

6.4 PACKAGING OF STUDY PRODUCT

All umbilical cord tissues will be assigned an ISBT Demand 128 bar code label or unique identifier, which is carried through to all in-process and final hCT-MSC products. In addition, the CT2 GMP facility will provide a final product label for each hCT-MSC product. The product label will include a space to affix the bar code label as well as space for the participant number, date and time of product expiry, and any other pertinent information. As a participant is enrolled, a participant number will be assigned which will link to the 12 digit ISBT number bar code number assigned to the umbilical cord blood tissue. The final product will be assigned a lot number (manufacturing operation number) and expiry date and time that will be denoted on the Certificate of Analysis and product label. The participant number and ISBT bar code number of the product will be also listed on the Certificate of Analysis. All products will be transported from the CT2 laboratory to the infusion center in a validated cooler by courier.

6.5 ADMINISTRATION OF PRODUCT

Patients will be admitted to the infusion center on the day of their scheduled infusion. Patients may require some sedation prior to the IV placement if they are unable to remain still or cooperate. A peripheral IV will be placed by clinical or study staff. Patients will be premedicated with Benadryl 0.5mg/kg/dose IV and Solumedrol 0.5-1mg/kg IV. The hCT-MSCs or placebo product will be administered intravenously over 30-60 minutes. Vital signs (heart rate, blood pressure, temperature, respiratory rate) will be attempted upon arrival to the clinic and monitored as clinically indicated. Pulse oximetry will be monitored continuously throughout the infusion and for at least 5 minutes post infusion. Patients should be observed a minimum of 15-30 minutes post infusion.

6.6 SAFETY FOLLOW-UP

On Day 1 following each infusion, the participant will be evaluated by study staff to assess for any infusion related adverse reactions or complications. This assessment can be done in person, over

the phone, by email or a teleconference. At 7-10 days post each infusion, a member of the study team will contact the parent or guardian via phone, teleconference, or email to assess patient status and any adverse events. A questionnaire (see Appendix 1) will be administered at each subsequent visit and at 3, 9 and 12 months to assess for serious adverse events. PRA will be obtained pre-treatment and at 6 and 12 months after the initial infusion.

7.0 STUDY PLAN

7.1 OVERVIEW

Parents/Guardians who have previously contacted our program and have a child who may meet eligibility criteria for this study will be notified that this study is available. After initial contact, parents/guardians of potential research participants will have an initial phone or teleconference interview with study personnel to describe the study, verify basic eligibility criteria, and confirm their interest in participation. The participant's eligibility will then be screened through review of medical and psychological records, therapy records, video, photos, cognitive and diagnostic testing, and laboratory testing under a separate screening protocol and consent.

Once all screening is complete and the participant is likely to meet study criteria, the participant will travel to Duke for their first visit. During their baseline visit at the Duke Center for Autism and Brain Development, the informed consent will be obtained and participant eligibility will be determined by physical observation and verification of ASD diagnosis per BOSA and DSM-5 criteria. During his/her first visit, he/she will also undergo additional clinical and observational evaluations, and SenseToKnow app assessments. Participants will be evaluated the day after infusion either in person, via teleconference, or by phone call and parents will be contacted 7-10 days after infusion for follow up safety evaluation. Participants will return to Duke six months later for repeated clinical and observational evaluations, and SenseToKnow app assessments, and safety follow-up. At 12 months post-infusion, remote safety assessment and parent-completed behavioral questionnaires will be completed.

7.2 PARTICIPANT SCREENING

Screening for the study is conducted under a separate, IRB-approved screening protocol (Pro00063563). Under this protocol, after written informed consent is obtained from a parent/guardian, the participant's medical and psychological records, therapy records, photographs, behavioral videos, and results of all genetic testing are obtained and reviewed by two teams. The medical review is conducted by a team of pediatric nurses, nurse practitioners, and physicians to identify the presence of any metabolic, immunologic, neurologic, sensory, genetic, or laboratory exclusion criteria. If no such exclusion criteria are identified, the psychiatric review is then conducted by a combination of psychologists, psychiatrists, and other clinically trained staff with expertise in diagnosing and treating children with ASD. They perform an extensive review of the participant's psychological records as well as any therapy records available. A participant must be approved by both medical and psychiatric screening teams to proceed with further laboratory or phone, teleconference, or in-person screening and study enrollment. Should a concern for a previously undiagnosed condition or genetic finding arise during the screening process, this will be discussed with the participant's parent(s)/guardian(s) and a referral will be made to an appropriate medical or psychiatric provider for evaluation and treatment, if indicated.

7.3 STUDY INFUSIONS

All participants will receive an infusion of allogeneic hCT-MSC cells. On the day of infusion, hCT-MSC cells will be thawed and prepared by the CT2 GMP laboratory and/or Duke Stem Cell Lab

per standard operating procedure and provided for infusion of the participant in the clinic under the supervision of the study team and Pediatric Blood and Marrow Transplant Program staff. Baseline vital signs (heart rate, blood pressure, temperature, respiratory rate) will be obtained. A peripheral IV will be placed by clinical staff, anesthesia or a member of the study team. Prior to the infusion, premedications (Benadryl, Solumedrol) will be administered. The study product will be infused over 30-60 minutes. The child will be observed in the clinic for a minimum of 30 minutes after the infusion. IV fluids (D5 ½ NS) at 1.5 maintenance will be attempted. Participants will be discharged from clinic after at least 1 hour providing all vital signs are at their baseline and they are asymptomatic with no evidence of toxicity. Participants will be evaluated by study staff the day after the infusion to assess for any infusion-related adverse reactions or complications. A phone call to parents/guardians by study staff to assess safety of the infusion will be conducted 7-10 days after the infusion.

If a participant has evidence of illness on the day of planned infusion, including but not limited to fever >38.5◦ C, vomiting, diarrhea, or respiratory distress, the infusion will be postponed.

7.4 CARE DURING UNEXPECTED EVENTS

In the event that a participant develops signs or symptoms of anaphylaxis including urticaria, difficulty breathing, cough, wheezing, or vomiting during his/her study infusion, the infusion will be interrupted and appropriate medical therapy initiated.

7.5 ASSESSMENTS

The following child assessments will be conducted at the Duke Center for Autism and Brain Development according to the schedule outlined in section 7.6:

Respondent	Measure	Domain	Length of Administration
Clinical Assessments	Brief Observation of Symptoms of Autism (BOSA)*	ASD Diagnosis	45-60 minutes
	Developmental Assessment of Young Children, Second Addition (DAYC-2) *	Cognitive/Language	45-60 minutes
	Clinical Global Impressions Scale – S and I	Autism symptoms	10 minutes
	JERI rating based on Parent-Child Interaction Task (PCIT)	Social communication	12 minutes
Biomarker Assessments			
	SenseToKnow (S2K) Application with Computer Vision Analysis (CVA)	Autism symptoms	10 minutes

*The DAYC-2 and BOSA are only conducted during screening

7.5.1 Assessments for Diagnosis of Autism Spectrum Disorder:

Diagnosis of ASD will be confirmed by the DSM-5 Checklist, which will be informed by the Brief Observation of Symptoms of Autism (BOSA) Assessment. Clinical research staff that have been certified as research reliable in the administration of the ADOS-2 will complete diagnostic evaluations.

Brief Observation of Symptoms of Autism (BOSA): This assessment will be used to help inform a DSM-5 diagnosis of ASD. The BOSA is a semi-structured parent-child interaction that lasts between 12 to 16 minutes. Age and verbal ability are used to determine the appropriate module of the BOSA. A clinician who is research-reliable on the ADOS-2 observes the BOSA and scores a corresponding ADOS-2 protocol. Following completion of the BOSA, an algorithm is used to map these binary codes onto a DSM checklist. The BOSA is only completed at the baseline visit.

Diagnostic Statistical Manual-5 Checklist (DSM-5): This is a clinician checklist based off of diagnostic criteria for ASD within the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. Diagnostic criteria are taken from the DSM-5, and are utilized in order to confirm appropriate ASD diagnosis for inclusion in the study. This checklist is completed by trained clinicians using the clinician's best judgment and is informed by information gathered during the administration of the BOSA. The DSM-5 Checklist takes about 3-5 minutes to complete. This checklist is completed at baseline.

7.5.2 Other Clinician Assessments:

Developmental Assessment of Young Children- Second Addition (DAYC-2): [92] The Developmental Assessment of Young Children (DAYC-2) is a cognitive based assessment designed to identify delays in very young children and preschoolers from birth through 5 years. Only the Cognitive scale will be administered. Test administration time varies from 10-20 minutes per domain. This assessment will be conducted during the baseline visit only.

SenseToKnow (S2K) Application (app): The application consists of a series of brief dynamic stimuli (including short, <1 minute movies and games) which are designed to elicit early risk behaviors related to autism. The application records the child's behavior via the front-facing camera as he or she interacts with the stimuli. Algorithms based on computer vision and machine learning are employed to quantify a multitude of autism symptom behaviors, including attentional responses, preferential gaze, facial affect, and motor responses. Dawson and Sapiro have conducted studies in in-clinic and at-home environments demonstrating that CVA can objectively and reliably quantify autism symptoms.[93-97] The application takes about 10 minutes will be conducted at the baseline and 6 month visits.

JERI Rating based on Parent-Child Interaction Task (PCIT): During a 12-minute Parent-Child Interaction Task (PCIT), the participant will be observed during two contiguous sessions conducted in the same room comprised of (1) a six-minute interactive play session in which the parent joins the child in play and (2) a six-minute independent play session with toys available during which the caregiver will be sitting in the corner of the room completing a questionnaire. The Parent-Child Interaction Task will be completed at the baseline and 6 month visits. Behavior will be video recorded with audio recording. Behavior will then be characterized through two methods: 1) automated video tracking with computer vision analysis, and 2) behavioral coding of child social communication abilities during interactive play using the Joint Engagement Rating Inventory (JERI).[91] Videotapes will be coded using JERI by trained and reliable raters. Computer vision analysis software will be used to automatically track the child's movements and the parent's movements. The purpose of the video tracking is to automatically measure social approach or avoidance and exploration. The primary dependent variable is the time spent in each core region (center of room, periphery of room, near parent, activity table). Exploratory dependent variables will be related to latency, frequency, velocity and movement.

The JERI will be used to code for child engagement states during the first 6-minute parent-child interactive play session. Behaviors that will be rated include: unengaged, object engaged, supported joint engagement, coordinated joint engagement, symbol-infused joint engagement, and total joint engagement. All items are rated on a 1 to 7 scale and factor in both the quantity and quality of each behavioral state of engagement. Dependent variables will include total joint engagement (primary endpoint) and ratings on all items (secondary endpoints).

Vineland Adaptive Behavior Scales, Third Edition (VABS-3) Survey Interview Form:[98] This will be used to assess social, communication, motor, and adaptive behavior. The assessment is administered to the parent/caregiver using a semi-structured interview format. This assessment

takes about 60-120 minutes to administer. The VABS-3 is a well-standardized measure of several domains of adaptive functioning including socialization, communication, daily living, and motor skills. Norms are available from birth to 90 years. The Socialization subdomain assesses play, interpersonal relationships and coping skills. The Communication subdomain assesses receptive, expressive and written language skills. The Daily Living Skills subdomain assesses personal, domestic and community living skills. The Motor Skills subdomain assesses gross and fine motor skills. The VABS-3 will be completed at the baseline, 6, and 12 months.

Clinical Global Impression (CGI): The CGI is a commonly used rating scale with two components that measures symptom severity and treatment response.

The Clinical Global Impression – Severity Scale (CGI-S) is a 7-point scale that requires the clinician to rate the severity of the participant's symptoms based on parent interview and child observation at the baseline and 6 month visits. The CGI-S requires the clinician to rate the severity of the participant's symptoms of ASD at the time of the assessment, relative to the clinician's past experience with participants who have the same diagnosis. There will be three separate CGI-S ratings; these include social communicative functioning, restricted/repetitive interests and behaviors, and overall. The CGI-S will be completed at the baseline and 6 months.

The Clinical Global Impression – Improvement (CGI-I) is a 7-point scale that requires the clinician to assess how much the participant has improved or worsened relative to baseline based on parent interview and child observation. There will be three separate CGI-I ratings; social communicative functioning, restricted/repetitive interests and behaviors, and overall improvement. The CGI-I will be completed at the 6-month visit.

7.5.3 Parent Caregiver Questionnaires:
All caregiver questionnaires will be completed online through a Duke approved EDC system or by paper. The EDC survey tool is available for Duke users through a university-wide site license. The EDC is integrated with Duke's NetID authentication system but allows sharing of surveys with non-Duke users.

Pervasive Developmental Disorder Behavior Inventory (PDDBI):[99] The PDDBI was developed to assess responsiveness to intervention in children with ASD. The PDDBI is an informant-based rating scale that is designed for children 1 year, 6 months to 12 years, 5 months. It assesses problem behaviors as well as appropriate social, language, and learning/memory skills. The PDDBI assesses both social impairments typically associated with the active but odd subtype of ASD and development of pro-social skills that are integral to improved reciprocal social behavior. The PDDBI renders raw scores as well as t-scores based on comparisons to a standardized ASD population. The PDDBI has been validated in a PDDBI development sample of 311 children between the ages of 1 and 17 years old. This is a parent questionnaire with 188 items that takes approximately 30-45 minutes to complete. The PDDBI will be administered every three months.

Intervention History Questionnaire: This questionnaire is completed by a primary caregiver to obtain detailed information on behavioral health interventions that the child/family has been involved in over the past 3 months or since the questionnaire was last administered. Information is collected about the type and quantity of interventions, services, and treatments the child is receiving. This questionnaire will be administered at baseline, 3 months and 6 months.

MacArthur-Bates Communicative Development Inventories, Second Edition (CDIs):[100]
The CDIs are standardized caregiver-completed report measures which capture information about children's developing abilities in early language, including gestures, production, vocabulary,

comprehension, and grammar. It was developed for use in infants and toddlers ages 8 to 30 months but it may also be used with older children with developmental delays. Because language changes so dramatically during that time span, there are three separate forms for different age ranges and language abilities. Each form generally takes 20-40 minutes to complete.

CDI: Words and Sentences form is designed for children ages 16 to 30 months. This form yields scores for vocabulary production and a number of aspects of grammatical development, including sentence complexity and the mean length of the child's longest utterances. The CDI words and sentences version will be used for all participants. This form will be collected at baseline, 3 months, 6 and 12 months.

Pediatric Quality of Life (PedsQL) 4.0 Generic Core Scales: The PedsQL 4.0 Generic Core Scales is a 5-minute parent questionnaire that measures the child's functioning in the dimensions of: physical, emotion, social and school. The Toddler Parent Report version is composed of 21 items and will be completed at the baseline and 6 month visits and remotely at 12 months.

Center for Autism and Brain Development (CABD) Demographics Form: The CABD demographics form collects race and ethnicity according to the NIH reporting standards. Additional questions collect data to help characterize the population. This parent questionnaire takes less than 10 minutes to complete and will only be completed at the baseline visit.

Medical and Behavioral History Questionnaire (MBHQ): The MBHQ is a broad assessment of medical and behavioral issues. It is completed at baseline, three, six, nine and 12 months.

COVID-19 Exposure and Family Impact Survey (CEFIS): [101] The CEFIS is an open-source parent report questionnaire that conceptualizes exposure to potentially traumatic aspects of COVID-19 and assesses the impact of the pandemic on the family. The CEFIS will be completed every three months. Data collected with the CEFIS measure will be de-identified and shared with the tool developers through a CSV file sent securely using DukeBOX.

7.6 REQUIRED EVALUATIONS

7.6.1 Medical and Safety Assessments:

	Time Points#				
	Screening	Baseline (Visit 1)	7-10 days post-infusion	6 months	12 months
CBCD*, CMP*, participant HLA, fragile X, CMA/WES	X				
Review of prior records ± videos	X				
History & Physical		X		X	
Samples for storage of DNA & viable mononuclear cells, DNA extract and hold		X			
Donor Referral Panel		X			
CBCD, CMP, HLA Antibody Screen (PRA), Immune Reconstitution Panel, Humoral Immune Profile		X*		X	X
Neuropsychological evaluation		X		X	X
Safety Assessment –in person (Day post-infusion)		X			
Safety Assessment – phone call/survey			X		

*CBCD, CMP, PRA may be obtained at initial visit or within 6 months prior to consent; HLA, fragile X, CMA/WES any time prior to enrollment.

Safety and return assessments should be performed within a month of the indicated time point.

7.6.2 Diagnostic, Behavioral, Cognitive/Language, and Attention evaluations.

	Measure	Time (min)	Baseline	3 mo.	6 mo.	9 mo.	12
Clinician Assessment w/ Child	**Brief Observation of Symptoms of Autism (BOSA)**	**45-60**	**X**				
	Developmental Assessment of Young Children – Second Edition (DAYC-2)	45-60	X				
Other Assessments w/ Child							
	SenseToKnow (S2K) app	10	X		X		
Observation of child	Parent-Child Interaction (PCI) for JERI rating	12	X		X		
Caregiver Interviews	Vineland Adaptive Behavior Scales, Third Edition Survey Interview Form	60-120	X		X		X
	Clinical Global Impression Parent Interview	30-45	X		X		
	Medical/Behavioral History Questionnaire (MBHQ)	15-30	X	X	X	X	X
Caregiver Questionnaires (Conducted with the Caregiver Only)	Pervasive Developmental Disorder Behavior Inventory (PDDBI)	30-45	X	X	X	X	X
	Intervention History	15	X	X	X	X	X
	MacArthur-Bates Communicative Development Inventories, Second Edition (CDIs)	20-40	X	X	X		X
	COVID-19 Exposure and Family Impact Survey (CEFIS)	15	X	X	X	X	X
	Pediatric Quality of Life (PedsQL)	5-10	X		X		X
	Demographic Form	10	X				
	Clinical Global Impression – Severity (CGI-S) Clinical Global Impression – Improvement (CGI-I)	15	X		X		
Other Clinician Completed	Diagnostic Statistical Manual-5 (DSM-5) Checklist	3-5	X				
	Clinical Global Impressions - Severity	3-5	X		X		

	Clinical Global Impressions - Improvement	3-5			X		

8.0 STATISTICAL CONSIDERATIONS

8.1 STUDY DESIGN

This is a single site, Phase I, prospective study of intravenous hCT-MSC infusion in 12 children 18 months to <4 years (3 years 364 days) of age with ASD. All participants will be treated with hCT-MSC. Participants will receive a single hCT-MSC infusion at baseline and evaluated at 6 (in person) and 12 (remotely) months post-infusion. Duration of study participation will be 12 months from the time of the first infusion.

8.2 STUDY DURATION

Research participants will be enrolled in the study for 12 months after the administration of the hCT-MSC infusion.

8.3 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline characteristics will be summarized for all research participants. Characteristics to be examined include age, sex, race/ethnicity, IQ, and baseline behavioral status.

8.4 SAMPLE SIZE AND POWER CALCULATIONS

The proposed trial is an open label Phase I trial of one intravenous infusion of hCT-MSC in toddlers 18 months to <4 years (3 years 364 days) of age with a diagnosis of ASD. The primary outcome is safety, as measured by the incidences of infusion reactions, infections, and new-onset clinically significant anti-HLA antibodies. Efficacy measures will also be assessed. The primary efficacy measure is coded time spent in sustained engagement with the caregiver in a brief semi-structured parent child interaction task (PCIT). The change on this outcome measure from Baseline to Month 6 will be analyzed.

8.5 PRIMARY ENDPOINTS

The primary outcome is safety. Safety endpoints will include the incidence of infusion reactions, infections, and new-onset clinically significant anti-HLA antibodies. Presence of infusion reactions and infections will be assessed in person at the time of infusion, 24 hours after infusion, and at six months and will be assessed remotely at 7-10 days post-infusion and 12 months and will be reported descriptively. Anti-HLA antibodies will be assessed at six and 12 months post-infusion.

8.6 EFFICACY ENDPOINTS

Primary Endpoint:

The primary clinical outcome measure is the change in social communication abilities from baseline to 6 months based on total score (1-7) on the Joint Engagement Rating Inventory (JERI), a commonly-used and well-validated coding system for rating the quality and quantity of social communication skills in toddlers with and without ASD.[91]

Secondary Endpoints:

Change measured on the following endpoints from baseline to six months will be compared:

1. PDD Behavior Inventory Autism Composite Score (PDDBI)
2. Mean of the Socialization Subscale and Communication Subscale standard scores on the Vineland Adaptive Behavior Scales (VABS-3)
3. Clinical Global Impression Scale (CGI) – Severity and Improvement (Improvement at 6 months only

4. Communicative Development Inventories (CDI-2): Words & Sentences subscales

Exploratory Endpoints*:*
Change measured from baseline to six months will be evaluated on the following outcome measures.

1. As measured by an app that elicits and records autism symptoms on an iPad (SenseToKnow), changes in auditory orienting, facial expressions, look duration, attention to social and nonsocial stimuli, postural sway, and vocalizations as measured via computer vision analysis

2. PDD Behavior Inventory (PDDBI) Subscales
3. VABS-3 Socialization Subscale Standard Score and age equivalent
4. VABS-3 Communication Subscale Standard Score and age equivalent
5. VABS-3 Daily Living Subscale Standard Score and age equivalent
6. VABS-3 Adaptive Behavior Composite Standard Score and age equivalent

9.0 SAFETY AND ADVERSE EVENT REPORTING

9.1 DEFINITIONS

Adverse Event (AE): An adverse event is any untoward medical occurrence associated with the use of the investigational product regardless of whether it is considered related to the investigational product.

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Grade/Severity: Grade/severity will be assessed according to CTCAE v5.0 guidelines.

Suspected Adverse Reaction: A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the investigational product and the adverse event.

Causality: The investigator will use the following question when assessing causality of an adverse event to the investigational product: "Is there a reasonable possibility that the investigational product caused the event?" An affirmative answer designates the event as a suspected adverse reaction.

9.2 ADVERSE EVENT REPORTING

All adverse events reported or observed during the study beginning at the time of the initial infusion must be recorded. AEs occurring in 5% of enrolled participants will be reported in clinicaltrials.gov. Information to be reported includes date when the site became aware of the event, investigator-specified assessment of severity and relationship to study therapy, whether there is an alternative etiology, seriousness, as well as any required treatment or evaluations, and

outcome. In general, investigators should report adverse events as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequelae.

Severe adverse infusion reactions (fatal, life-threatening or requiring hospitalization) will be reported within seven calendar days of receipt of the information. All fatal or life threatening SAEs will be reported by the investigator or its representatives to the FDA by telephone or fax within seven calendar days after receipt of the information, following FDA guidelines. All serious and unexpected AEs will be reported to the FDA via a written report within 15 days of receipt of the information (21 CFR 312.32). If the principal investigator assesses an event to be unrelated to the study, then the event will not require expedited reporting but will be included in the annual summary report.

The following events within 24 hours of hCT-MSC infusion will also be recorded in the e-CRF: infusion reaction, sinus bradycardia, sinus tachycardia, hypertension, hypotension, fever, rigors/chills, nausea, vomiting, infection, dyspnea, hypoxia, and hemoglobinuria.

9.3 SERIOUS ADVERSE EVENT REPORTING

The Principal Investigator or its representative will be responsible for telephone or fax reporting of any unexpected SAEs to the FDA. The Principal Investigator or its representative will notify the FDA by telephone or fax of any fatal or life threatening experience (expedited report) associated with the use of the study therapy as soon as possible but no later than seven calendar days after receipt of the information. Initial notification will be followed by a written report within 15 calendar days. For SAEs associated with the use of the study therapy, the Principal Investigator will notify the FDA as soon as possible, but no later than 15 days, of the initial receipt of the information. The Principal Investigator or Sub-Investigator is responsible for informing the Institutional Review Board (IRB) and DSMB of any study related and unexpected SAEs.

9.4 ELICITING ADVERSE EVENT INFORMATION

In addition to research participant observations, AEs will be documented from any data collected throughout the study including clinically significant laboratory values or physical exam findings.

9.5 STOPPING GUIDELINES

The following stopping guidelines will be monitored during the duration of the study. The stopping guidelines will be monitored by the study team and are to be used to indicate boundaries requiring discussion by the investigators and DSMB. The study will be stopped for a safety review if:

- Any participant experiences a grade 4-5 infusion reactions within 48 hours of infusion; OR
- Two or more grade 4-5 adverse events determined to be temporally related to the study product by the medical safety monitor and/or the DSMB occur;
  OR
- Any participant experiences a blood stream infection within 6 months of infusion;
  OR
- Any participant develops grade II-IV GvHD;
  OR
- Any death.

A consensus decision to stop the study will be made by the investigators and the DSMB. Such a decision with its supporting documentation and possible future plans for the study will be submitted to, and discussed with, the FDA.

9.6 PARTICIPANT REPLACEMENT

In the event that any enrolled participants do not receive treatment, additional participants may be randomized in order to reach the target number of evaluable, treated participants.

10.0 DATA SAFETY MONITORING BOARD (DSMB)

A DSMB will be formed and a charter established. Members of the DSMB will be independent of Duke University and comprised of a minimum of three members, including a clinician with experience in the treatment of ASD and a physician with experience in cell therapy. The DSMB will be notified immediately for all SAEs directly related to the study product throughout the study. A total safety assessment will be prepared on an annual basis and forwarded to the DSMB for review as well. The DSMB and its members will have the right to make decisions and act independently. Policies of the DSMB will be described in the DSMB charter and signed by all members.

All study related and unexpected SAEs reported or observed during the study beginning at the time of the study infusion must be recorded and maintained in the study participant's paper files. Severe adverse infusion reactions (fatal, life threatening or requiring hospitalization) will be reported to the IRB and FDA in accordance with HRPP policies.

11.0 DATA HANDLING AND QUALITY ASSURANCE

11.1 CASE REPORT FORMS

As part of the responsibilities assumed by participating in the study, the Principal Investigator or Sub-Investigators agree to maintain adequate case histories of the research participants treated as part of the research under this protocol. The Principal Investigator or Sub-Investigator agrees to maintain accurate CRFs and source documentation as part of the case histories. Duke University will supply the CRF electronically (eCFR) through secured electronic data entry systems.

11.2 VIDEO AND AUDIO RECORDINGS

Video recordings of potential participants from parents and guardians may be submitted and used for determining study eligibility. Audio and video recordings may also be obtained of portions of the evaluations and interviews if indicated with parental consent, and may include full facial features and audio. The recordings will be used solely for analysis by the research team or for educational purposes if consent is obtained from the parent/guardian. They will be stored electronically on a password-protected server and identified by the participants' study ID. Software used to conduct and record visit assessments completed remotely will be done on Duke approved platforms and participants will receive instructions to enhance privacy and security measures.

11.3 INSPECTION OF RECORDINGS

The Principal Investigator or Sub-Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the Principal Investigator or Sub-Investigator agrees to allow the Food and Drug Administration (FDA) or other regulatory agency access to all study records. The Principal Investigator or Sub-Investigators should promptly notify all relevant parties of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the both.

11.4 STUDY RECORD RETENTION

Study results will be retained in the participant's research record for six years after the study is completed or until the participant reaches the age of 21, whichever is longer. Essential documents should be retained until at least two years after the last approval of a marketing application in an International council for Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements.

11.5 Process for adding the Study Diagnostic Report to Patient's Duke Medical Record

As part of this study, it the participant (or their LAR, as applicable) also receives clinical services at Duke outside of the study, they may request have their clinical evaluation report(s) added to their Duke Maestro chart. Doing this will allow researchers and clinicians at the Duke Center for Autism and Brain Development to better integrate our research and clinical programs. If the patient is getting care outside of the study at Duke, they may benefit from this by making clinical evaluations and care in the non-research context more seamless, allowing providers to review reports from clinical assessments conducted as part of the study in order to inform clinical care, and avoiding unnecessary duplication of efforts on the part of both research participant and study team. Most participants of the study are not patients receiving regular medical care at Duke outside of the study. However, in the event that a participant would like to have the report added to the medical record, they may make a request to do so.

When receiving a request, the study team will let the participant know, or remind them, that the diagnostic report will summarize diagnoses, developmental function, and cognitive skills and other symptoms and abilities that were assessed as part of the study; that we will not "list" or change diagnoses in the medical record, but will make the report available for other Duke Health providers to review. We will also note that the report will indicate that the individual received the evaluation as part of a study. Documentation of requests will be stored with the participant's research record.

11.6 Participant Engagement

Participants of this study may be contacted after their study participation to share their perspective and opinions on their study experience, including any parts that they enjoyed and/or any challenges. They will be reassured that this is completely optional. Questions include: their experience in the study generally, their opinion on research study assessments, the quality of print materials and interactions with our staff, anything about the study they found helpful and any challenges encountered, and what changes might be helpful to consider in the future. Interviews may be recorded on Duke-approved platforms if the family agrees. These open-ended questions and responses will not be asked as a part of this study, or used for data analysis, or to make conclusions about the study, but may be used in efforts to support the Duke Center for Autism and Brain Development's efforts in connecting with the broader community via social media, conferences, newsletters, seminars and other events/media about our research efforts and impact at the Duke Center for Autism. This activity will be key to the Center's efforts in promoting active engagement and bidirectional exchange with the families we serve and the broader community. These interviews may also help inform designing protocols and/or making changes as needed to enhance inclusivity, cultural appropriateness, and feasibility. If staff perform post-study interviews, participants will be asked whether they want to publish their identity in any publicly published information or would prefer to not be named. The relevant information will be added to a HIPAA waiver provided by the News office and authorization will be obtained through signing of the HIPAA waiver. Although these interviews and dissemination and outreach core (DOC) activities are not part of the study, we wish to inform the IRB that these interactions may take place as it may entail additional interactions with research participants.

Additionally, participants who are willing to have their image(s) or video(s) (including voice) used for purposes of media, news, trainings, articles, conferences and other dissemination activities, will also be asked to sign the appropriate HIPAA waiver provided by the DUHS News office with their selections and permissions noted in the form.

12.0 ADMINISTRATIVE ASPECTS

The following administrative items are meant to guide the Principal Investigator or Sub-Investigator in the conduct of the study but may be participant to change based on industry and government Standard Operating Procedures or Working Practice Documents or Guidelines.

12.1 CONFIDENTIALITY

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain research participant confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the research participant's guardian except as necessary for monitoring and auditing.

The Principal Investigator or Sub-Investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study.

12.2 INSTITUTIONAL REVIEW BOARD APPROVAL

Federal regulations and the ICH guidelines require that approval be obtained from an IRB prior to participation of human research participants in research studies. Prior to the study onset, the protocol, informed consent, any advertisement used to recruit study participants, and any other written information regarding this study to be provided to the research participant or the research participant's legal guardian must be approved by the IRB.

All IRB approvals should be signed by the IRB Chairman or designee and must identify the IRB name and address, the clinical protocol by title and/or protocol number, and the date the approval and/or favorable opinion was granted.

The Principal Investigator or Sub-Investigator is responsible for obtaining continued review of the clinical research at intervals not exceeding one year or otherwise specified by the IRB. The Principal Investigator or Sub-Investigator must supply the Sponsor or its designee with written documentation of continued review of the clinical research.

12.3 MODIFICATION OF THE PROTOCOL

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the research participant, must be reviewed and approved by the IRB.

12.4 INFORMED CONSENT

A written informed consent in compliance with Part 50 of Title 21 of the Code of Federal Regulations (CFR) and Institutional IRB shall be obtained from each research participant prior to entering the study or performing any unusual or non-routine procedure that involves risk to the research participant.

Before enrollment, each prospective research participant and/or at least one legal guardian will be given a full explanation of the study and be allowed to read the approved informed consent form.

Once the Principal Investigator or Sub-Investigator is assured that the research participant/legal guardian understands the implications of participating in the study, the research participant/legal guardian will be asked to give consent to participate in the study by signing the informed consent form.

The Principal Investigator or Sub-Investigator shall provide a signed/dated copy of the signed informed consent to the research participant and/or legal guardian.

12.5 PROTOCOL VIOLATIONS AND DEVIATIONS

The Principal Investigator or Sub-Investigator or designee must document and explain in the research participant's source documentation any deviation from the approved protocol. The Principal Investigator or Sub-Investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study research participants without prior IRB approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendment(s) should be submitted to the IRB for review and approval, to the Sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the Sponsor and the IRB and agreed to by the Principal Investigator or Sub-Investigator. Deviations usually have an impact on individual research participants or a small group of research participants and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when there is non-adherence to the protocol that results in a significant, additional risk to the research participant, when the research participant or Principal Investigator or Sub-Investigator has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the research participant was enrolled without prior Sponsor approval, or when there is non-adherence to FDA regulations and/or ICH GCP guidelines.

Protocol violations and deviations will be documented by the study coordinator and the clinical monitor will notify study staff and update protocol deviation documentation throughout the course of monitoring visits. Principal Investigators or Sub-Investigators will be notified of violations and/or deviations in writing by the study coordinator and/or the study monitor. The IRB should be notified of all protocol violations and deviations meeting criteria in a timely manner as required by the site's IRB.

12.6 STUDY REPORTING REQUIREMENTS

By participating in the study, the Principal Investigator or Sub-Investigator agrees to submit reports of serious adverse events according to the timeline and method outlined in the protocol. In addition, the Principal Investigator or Sub-Investigator agrees to submit annual reports to his/her IRB as appropriate. The Principal Investigator or Sub-Investigator also agrees to provide the Sponsor with an adequate report shortly after completion of the Principal Investigator's or Sub-Investigator's participation in the study.

12.7 FINANCIAL OBLIGATIONS

Duke University is not financially responsible for further testing/treatment of any medical condition that may be detected during the screening progress. In addition, in the absence of specific arrangements, Duke University is not financially responsible for further treatment of the research participant's disease.

12.8 STUDY CONDUCT

The Principal Investigator agrees that the study will be conducted according to the principles of the ICH E6 Guideline on GCP and the principles of the World Medical Association Declaration of Helsinki. The Principal Investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

12.9 PUBLICATIONS

Following completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, Duke University will be responsible to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues.

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14.0 APPENDIX 1: SAFETY QUESTIONNAIRE

BASELINE MEDICAL & BEHAVIORAL HISTORY QUESTIONNAIRE

Please tell us if your child has ever experienced any significant problems with any of the following conditions using the column marked EVER. If so, tell us whether they have continued to experience significant problems with that condition **during the past 2 months** in the next column.

		EVER		Past 2 months	
		No	Yes	No	Yes
1	Has your child ever had any fevers with no clear cause?				
2	Has your child ever had any serious or recurrent infections?				
3	Has your child ever had any allergic reactions?				
4	Has your child ever had significant problems with autoimmune disorders?				
5	Has your child ever had any significant problems with rashes or abnormal skin lesions?				
6	Has your child ever had jaundice (yellowing of the skin or eyes), except as a newborn?				
7	Has your child ever received a blood transfusion?				
8	Has your child ever had any significant problems with bloody noses, easy bruising or prolonged bleeding?				
9	Has your child ever had any abnormal blood tests?				
10	Has your child ever had any problems with breathing, asthma or coughing?				
11	Has your child ever had any problems that make you concerned about his/her heart?				
12	Has your child ever had any significant problems with his/her mouth or teeth?				
13	Has your child ever had any bowel problems, including any bowel accidents?				
14	Does your child have ongoing problems with diarrhea?				
15	Has your child ever had any ongoing episodes of vomiting?				
16	Has your child ever had any significant problems with appetite?				
17	Has your child ever had any significant problems with weight?				
18	Has your child ever had any significant problems with his/her muscles or joints?				
19	Has your child ever had any problems with swelling in any part of his or her body?				
20	Has your child ever had any problems with his/her urinary tract or problems when he/she urinates?				
21	Has your child ever had any problems with his/her breasts/nipples or private parts?				
22	Has your child ever had any problems with how thirsty he or she is?				
23	Has your child ever had any problems with his/her ears or hearing?				
24	Has your child ever had any eye or vision problems?				
25	Has your child ever had problems with severe or frequent headaches?				

		EVER		Past 2 months	
		No	Yes	No	Yes
26	Has your child ever had any significant problems with dizziness?				
27	Has your child ever had any seizures?				
28	Has your child ever had any problems with involuntary movements?				
29	Is your child chronically tired or fatigued?				
30	Has your child ever had cancer?				
31	Has your child ever had a tumor?				
32	Has your child ever had any unexplained masses or growths?				
33	Have you ever had any other concerns about your child's medical health? If so, please explain below.				
34	Has your child ever had any significant problems sleeping at night?				
35	Has your child ever had any significant problems being too sleepy during the day?				
36	Has your child ever had differences in sensory processing such as sensory seeking behaviors or sensory aversion behaviors (including food aversion)?				
37	Has your child ever had any problems with rituals, being flexible, repetitive behaviors or repetitive language?				
38	Has your child ever had any problems with hyperactivity or impulsivity?				
39	Has your child ever had any significant problems with refusing to follow directions that he/she understands?				
40	Has your child ever had any significant problems with aggression, irritability or getting frustrated easily?				
41	Has your child ever had significant problems (more frequent/severe than other kids) with meltdowns or agitation?				
42	Has your child ever had any problems with staying motivated?				
43	Has your child ever had ever any problems with worries or sadness?				
44	Has your child ever had any problems with hurting him/herself on purpose or wanting to die?				
45	Has your child ever had any significant problems with mood swings?				
46	Has your child ever had any problems with believing things that aren't true or seeing/hearing things that aren't there?				
47	Has your child ever had any problems with seeming not to know where he/she was or what was really happening?				
48	Have you ever had any other concerns about your child's behavior, which is not addressed in questions 33-45? If so, please explain below.				

Please use the space below to explain any "yes" answers.

FOLLOW-UP MEDICAL & BEHAVIORAL HISTORY QUESTIONNAIRE

Please tell us if your child has ever experienced any significant problems with any of the following conditions SINCE YOU LAST COMPLETED THIS QUESTIONNAIRE.

		Past 3 months	
		No	Yes
1	Has your child had any fevers?		
2	Has your child had infections?		
3	Has your child had any allergic reactions?		
4	Has your child had any new or worse problems with autoimmune disorders?		
5	Has your child had any new rashes or abnormal skin lesions?		
6	Has your child developed jaundice (yellowing of the skin or eyes)?		
7	Has your child received a blood transfusion?		
8	Has your child had any new problems with bloody noses, easy bruising or prolonged bleeding?		
9	Has your child had any abnormal blood tests since their last visit?		
10	Has your child had any new or worsening problems with breathing, asthma or coughing?		
11	Has your child had any new or worsening problems with their heart?		
12	Has your child had any new problems with his/her mouth or teeth? (Do not include losing baby teeth.)		
13	Has your child had any new or worsening bowel problems, including bowel accidents?		
14	Has your child had any new or worsening diarrhea?		
15	Has your child had any new or worsening episodes of vomiting?		
16	Has your child had any new or worsening problems with appetite?		
17	Has your child had any new or worsening problems with weight?		
18	Has your child had any new or worsening problems with his/her muscles or joints?		
19	Has your child had any new or worsening swelling in any part of his or her body?		
20	Has your child had any new or worsening problems when he/she urinates?		
21	Has your child had any new or worsening problems with his/her breasts/nipples or private parts?		
22	Has your child had any changes in thirstiness?		
23	Has your child had any new or worsening problems with his/her ears or hearing?		
24	Has your child had any new or worsening eye or vision problems?		
25	Has your child had any new or worsening headaches?		
26	Has your child had any new or worsening problems with dizziness?		
27	Has your child had any new or worsening seizures?		
28	Has your child had any new or worsening problems with involuntary movements?		
29	Has your child had any new or worsening chronic tiredness or fatigue?		

		Past 3 months	
		No	Yes
30	Has your child ever had cancer?		
31	Has your child ever had a tumor?		
32	Has your child ever had any unexplained masses or growths?		
33	Has your child been hospitalized? If so, please explain below.		
34	Has your child had any new significant injuries?		
35	Have you ever had any other concerns about your child's medical health? If so, please explain below.		
36	Has your child had any new or worsening problems sleeping at night?		
37	Has your child had any new or worsening problems being too sleepy during the day?		
38	Has your child had any new or worsening problems with differences in sensory processing such as sensory seeking behaviors or sensory aversion behaviors (including food aversion)?		
39	Has your child had any new or worsening problems with rituals, being flexible, repetitive behaviors or repetitive language?		
40	Has your child had any new problems with hyperactivity or impulsivity?		
41	Has your child had any new or worsening problems with refusing to follow directions that he/she understands?		
42	Has your child had any new or worsening problems with aggression, irritability or getting frustrated easily?		
43	Has your child had any new or worsening with meltdowns or agitation?		
44	Has your child had any new or worsening problems with staying motivated?		
45	Has your child had any new or worsening problems with worries or sadness?		
46	Has your child had any new or worsening problems with hurting him/herself or wanting to die?		
47	Has your child had any new or worsening problems with mood swings?		
48	Has your child had any new or worsening problems with believing things that aren't true or seeing/hearing things that aren't there?		
49	Has your child had any new or worsening problems with knowing where he/she is or who is with him/her?		
50	Has your child had any new or worsening problems with their thinking?		
51	Have there been any other new or worsening problems with your child's behavior, which is not addressed in questions 35-50? If so, please explain below.		

Please use the space below to explain any "yes" answers.