2017 Review: Progress in Using Human Stem Cells in the Treatment of Autism Spectrum Disorder

AUTHOR

Panama College of Cell Sciences, Azuero Business Center, Suite 758
Avenida Perez Chitre, 00395, Panama
Republica de Panama

Abstract: *Autism and Autism Spectrum Disorders are a set of heterogeneous and enigmatic neurodevelopmental pathologies that arise from a variety of triggers. In spite of outstanding scientific achievements in the study of the pathologies associated with autism and autism spectrum disorders, as of 2017 these developmental disorders are still without a curative treatment option. This analysis reviews the therapeutic characteristics of stem cells and how they can provide clinical application and novel treatment options for autism spectrum disorders. This review integrates a concise evaluation of all stem cell types utilized in autism research and treatment and associates the efficacy, safety, and tolerability findings of the most current stem cell-autism spectrum disorder treatments. Concluding data outlines budgetary and funding rates associated with stem cell-autism spectrum disorder research (2012-2017) and concludes with avenues for novel research and treatment options.*

Keywords: stem cell, autism, autism spectrum disorder, umbilical cord, stem cell treatment, mesenchymal stem cell

Introduction

Autism and Autism Spectrum Disorders (ASDs) are a set of heterogeneous and enigmatic neurodevelopmental pathologies that arise from genetic susceptibility, environmental triggers, and epigenetic processes activated by those environmental triggers. As defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), published by the American Psychiatric Association, Autism Spectrum Disorder is categorized in rank of severity (Table 1) and characterized by persistent deficits in both social-emotional reciprocity and verbal and nonverbal communicative behaviors. Additional social and behavioral symptoms of ASDs include stereotyped or repetitive motor movements and insistence on sameness.

In review of the physiologic underpinnings of ASDs, it is found that several biochemical and cellular events are associated with ASDs: oxidative stress, endoplasmic reticulum stress, decreased methylation capacity, limited production of glutathione, mitochondrial dysfunction, intestinal dysbiosis and inflammation, increased toxic metal burden, impaired detoxification, and dysregulation of the brain's intrinsic immune system - including autoimmunity and activation of neuroglial cells (Bradstreet, Smith, et al, 2010; Ming, Brimacombe, et al, 2008). Despite this extensive body of evidence for an underlying immunotoxicological event in the development of autism, the exact origins of pathogenesis and pathophysiology of ASDs remain to be fully elucidated (Siniscalco, Bradstreet, et al, 2013).

The prevalence of ASDs is rapidly increasing. The DSM-5 report frequencies for ASDs approaching 1% of the population while the Centers for Disease Control and Prevention (CDC) report that 1 in 68 children are diagnosed with ASDs (reported in 2012 with a birth year of 2004). This is a change that cannot be explained by mere data-gathering methods, nor is it likely due to changed diagnostic approaches (Baio, 2012).

In spite of outstanding achievements in the study of pathogenesis of autism and other ASDs, including immunological and immunegenetic research resulting in the new trends of specific therapy, autism remains one of the unresolved issues of modern neurology (Bradstreet, Sych, et al, 2014).

Literature elucidates the current facets of ASD treatment and divides them into behavioral, nutritional, and biomedical approaches, with no defined 'gold-standard' existing. ASD pharmacologic therapies only target specific limited behavioral symptoms (Figure 1) and fail to resolve the basic underlying biological etiologies. Current research suggests that stem cell (SC) therapies represent the future of molecular and regenerative medicine for what would otherwise be untreatable human diseases. Stem cells are also suitable for developing cell-based patient-specific pharmacotherapies (Siniscalco, Giordano, et al, 2012; Siniscalco, Pandolfi, et al, 2012). These characteristics suggest that stem cells can provide therapeutic applications and new treatment options for ASDs.

Past studies and reviews have independently delineated various stem cell types used in the treatment of ASDs. There have also been independent studies which demarcate Stem Cell-Autism Spectrum Disorder (SC-ASD) treatment protocols and ongoing SC-ASD treatment options however, no review has ever encompassed all of these topics while also including examples of treatment centers that offer the most current SC-ASD treatment options.

This analysis proves to be a novel approach in that it will incorporate multiple data points into one study. This review integrates a concise evaluation of all SC types utilized in ASD research and treatments (as of 2017) and associates the efficacy, safety, and tolerability findings of the most current SC-ASD treatments. Also included in this analysis are the budgetary and funding rates associated with SC-ASD research (2012-2017) and avenues for new SC-ASD research and treatment. The review concludes with delineated options of treatment centers that offer the most advanced SC-ASD therapies.

Table 1:

Severity Levels for Autism Spectrum Disorder

Severity level	Social communication	Restricted, repetitive behaviors
Level 3 "Requiring very substantial support"	Severe deficits in verbal and nonverbal social communication skills cause severe impairments in functioning, very limited initiation of social interactions, and minimal response to social overtures from others. For example, a person with few words of intelligible speech who rarely initiates interaction and, when he or she does, makes unusual approaches to meet needs only and responds to only very direct social approaches	Inflexibility of behavior, extreme difficulty coping with change, or other restricted/repetitive behaviors markedly interfere with functioning in all spheres. Great distress/difficulty changing focus or action.
Level 2 "Requiring substantial support"	Marked deficits in verbal and nonverbal social communication skills; social impairments apparent even with supports in place; limited initiation of social interactions; and reduced or abnormal responses to social overtures from others. For example, a person who speaks simple sentences, whose interaction is limited to narrow special interests, and how has markedly odd nonverbal communication.	Inflexibility of behavior, difficulty coping with change, or other restricted/repetitive behaviors appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. Distress and/or difficulty changing focus or action.
Level 1 "Requiring support"	Without supports in place, deficits in social communication cause noticeable impairments. Difficulty initiating social interactions, and clear examples of atypical or unsuccessful response to social overtures of others. May appear to have decreased interest in social interactions. For example, a person who is able to speak in full sentences and engages in communication but whose to- and-fro conversation with others fails, and whose attempts to make friends are odd and typically unsuccessful.	Inflexibility of behavior causes significant interference with functioning in one or more contexts. Difficulty switching between activities. Problems of organization and planning hamper independence.

*data obtained from DSM-5

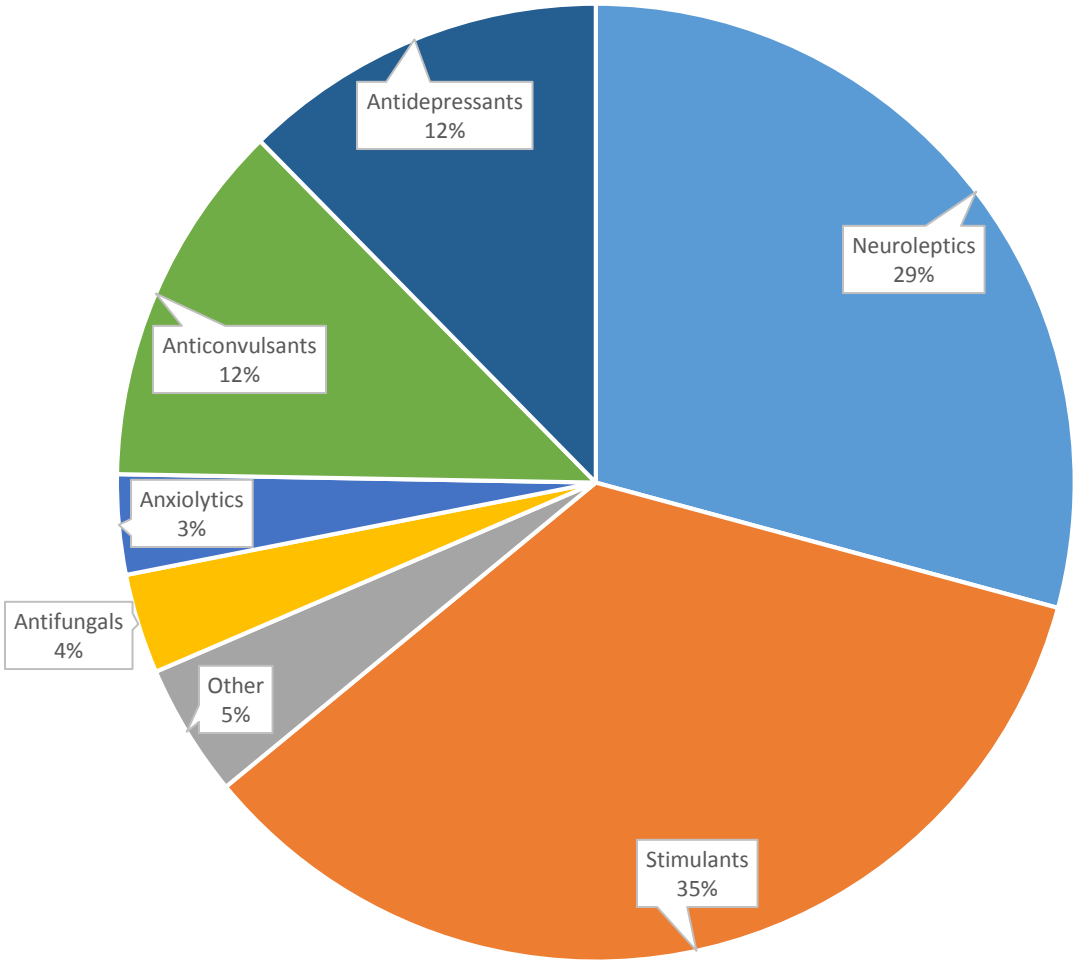

Figure 1. *Categories of Medications Used by Children with ASD.* Most of these medications have not been approved by the United States Food and Drug Administration (FDA) to treat ASD in adults or children however, there is evidence in research literature that supports their use in ASD. Stimulants treat: distractibility, attention deficits, and hyperactivity. Neuroleptics (i.e.: antipsychotics) treat: irritability, tantrums, aggression, and unstable mood. Antidepressants treat: stereotypic (repetitive) behaviors, unstable mood, anxiety, and depression. Anticonvulsants treat: unstable mood, seizures (epilepsy), and migraine headache. Anxiolytics treat: anxiety. Antifungals (e.g.: Diflucan) are used to treat the overgrowth of yeast-like fungus. Some clinicians believe that symptoms of ASD are exacerbated by the overgrowth of these fungi. Other medications in the study included antibiotics, antihistamines, and laxatives. These data include an N of 3,140 medications. These values are based on Interactive Autism Network (IAN) data collected on March 20, 2008.

Methods

The aim of this review was to define the progress in using stem cells in the treatment of autism spectrum disorders. In order to ensure a precise analysis, multiple research methods were implemented in order to: define the types of stem cells used in treating ASDs, establish efficacy in the treatments, and identifying appropriate treatment facilities.

First, a comprehensive evaluation of peer-reviewed journals was conducted. Key terms utilized during this search included: stem cells, autism, autism spectrum disorders, cell biology, pediatrics, cell, brain. Secondly, a comprehensive appraisal of peer-reviewed journal *articles* was conducted. Key words associated with this search included: treatment, stem cell treatment, autism, autism spectrum disorder. One U.S. Governmental database was used during these journal article and journal searches: ncbi.nlm.nih.gov (PubMed). In addition to the compilation of a journal articles, the reference section for each article chosen was reviewed in order to locate supplementary useful articles.

Current diagnostic medical manuals were utilized to ensure appropriate diagnostic criteria and statistics.

In order to ensure current research and funding data were obtained, multiple governmental, academic, and research institutional websites were utilized: www.cdc.gov, clinicaltrials.gov, autismcenter.duke.gov., cellmedicine.com, stemcelltreatmentnow.com.

Five International treatment centers were reviewed via emails, phone calls, and web searches. Final data collection methods included interviews with patients and families associated with SC-ASD treatments.

All defined research methods were conducted in such a manner to ensure data was reflective of 2017 outcomes.

Results

Although autism and ASDs are characterized by a variety of deficits in both social-emotional reciprocity and verbal and nonverbal communicative behaviors, there are two common consistent pathologies associated with children diagnosed with ASDs. The first of these pathologies is neural hypoperfusion. Multiple areas of the brain are affected in ASDs (Figure 2) and when these regions experience diminished oxygenation the resultant effect is cerebral ischemia. The second common pathology associated with ASD is immune dysregulation. This pathology can present as; a chronic immunologically medicated inflammatory condition in the gut, an upregulation of inflammatory cytokines in the ASD brain, and as alterations in immune cells such as T cells, B cells, monocytes, natural killer cells, and dendritic cells. (Riodan, 2016; Noriega, Savelkoul, 2014; Bjorklund, Saad, et al, 2016). These neural and immune dysregulations provide specific targets for specific stem cell therapies.

Stem cells possess several useful characteristics which suggests their potential therapeutic application for ASDs. Siniscalco and colleagues (2013) report that these characteristics are (1) their self-renewal ability: stem cells are able to generate more identical stem cells; (2) differentiation process: through it, the cells give rise to more differentiated cells; and (3) paracrine regulatory functions: stem cells synthesize and release a complex and implantable "biopharmacy", capable of regulating cell differentiation, tissue and organ repair, and anti-inflammatory actions in the recipient. These paracrine functions of stem cells (i.e.: the biopharmacy or the secretome) are attracting much attention (Razavi, Razavi, et al, 2013; Drago, Cossetti, et al, 2013). It has already been proposed that in ASD cell-based treatment, the positive effects that could be medicated by stem cells could be achieved through the trophic and immunomodulatory properties (Siniscalco, Sapone, et al, 2012). Additional research suggests that implanted stem cells (whether autologous or donor) are able to affect the recipient immune system through two proposed mechanisms: (1) cell-to-cell contact activation mechanism, through which transplanted stem cells switch proinflammatory macrophages to anti-inflammatory macrophages (Zheng, Ge, et al, 2013; Siniscalco, Giordano, et al, 2011), and (2) the paracrine-secretome activity (Zemel'ko, Kozhukharova, et al, 2013). Siniscalco, and colleagues (2013) propose that through these mechanisms, stem cells could simultaneously counterbalance the immune system aberrations while activating endogenous restorative mechanisms within damaged tissues contributing to recovery of functional deficits. It has also

been hypothesized that cell replacement, by transplanted stem cells, might not be a necessary prerequisite for effective stem cell therapies due to the aforementioned mechanisms proving to be sufficiently restorative however, these restorative functions are dependent upon the stem cell type and the programming of the stem cell.

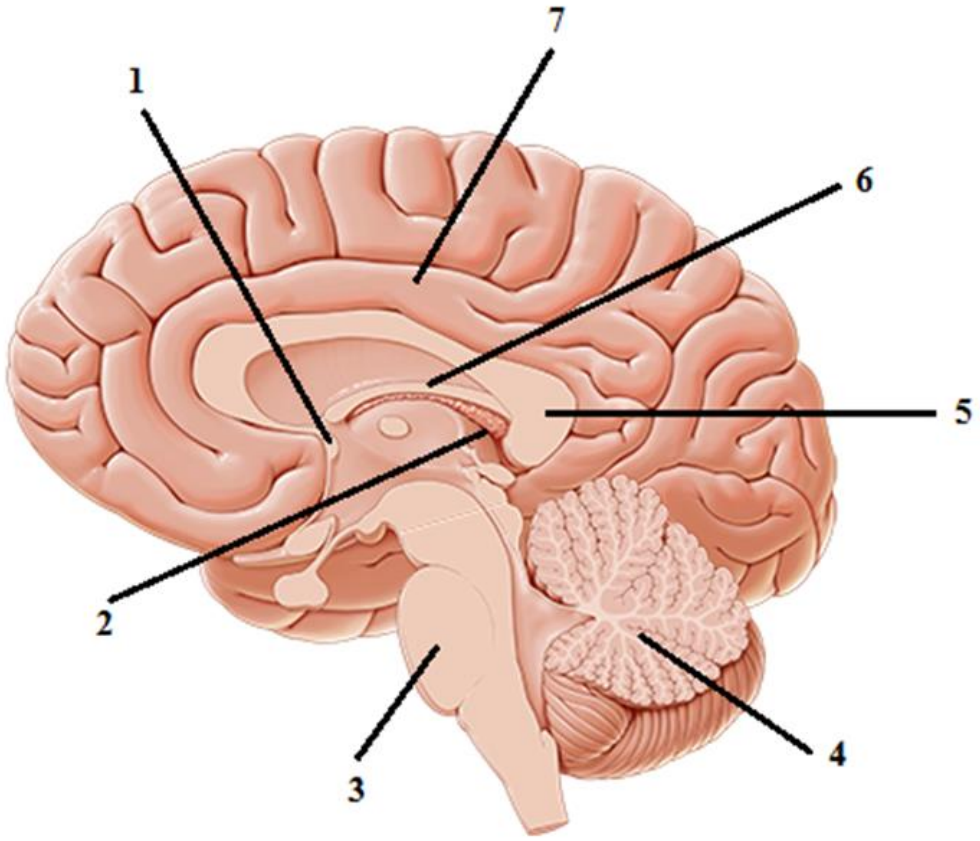

Figure 2. *Areas of the Brain Affected by Autism* (1) *Amygdala*: responsible processing emotions and behavior (including aggression); (2) *Hippocampus*: involved in learning and memory; (3) *Brain Stem*: serves as a relay link passing messages between the body and the cerebral cortex. Also controls the primitive functions of the body which ae essential to survival (breathing, heart rate); (4) *Cerebellum*: concerned with coordination and control of voluntary muscular activity; (5) *Corpus Callosum*: white-matter bridge between the two hemispheres of the brain – allowing the two hemispheres to communicate with one another; (6) *Basal Ganglia*: masses of gray-matter-lining in the brains cerebral cortex and involved in the control of automatic body movement; (7) *Cerebral Cortex*: thin layer of gray matter, two-thirds of this area is deep in the tissues and folds of the cerebrum. This is the center for higher mental functions, perception, behavioral responses, and other functions.

As of 2017 a variety of stem cell types have been considered and/or utilized in such stem cell therapies, which have resulted in varied outcomes.

Human Embryonic Stem Cells

Human Embryonic Stem Cells (hESCs) are derived from the inner cell mass (ICM) of the blastocyst (early-stage preimplantation embryo). These hESCs are known to be pluripotent which gives them the capability of differentiating into any of the three germ layers (i.e.: ectoderm, mesoderm, and endoderm). However, the utilization of hESCs in the clinical setting are plagued with numerous disadvantages, one of which is that of histocompatibility (i.e.: graft-versus-host-disease). Tang, Weissman, and colleagues (2013) go on to confirm that in the absence of a life-long regimen of antirejection medications, the cells would be expected to be rejected by the recipient immune system once HLA-II expression occurred. Furthermore, the implantation of hESCs into a recipient have been known to produce tumor-like formations (teratoma) which contain tissues from all three germ layers. The hESCs ability to form teratoma is a sine qua non characteristic of pluripotent stem cells (Prokhorova, Harkness, et al, 2009). Siniscalco and colleagues (2013) confirm that the proper regulation of post-transplantation of hESCs is the formidable challenge and that hESCs are known to derive their differentiation characteristics from the recipient environment. These challenges of chimeric engraftment are far from understood in the pediatric population, and thus the potential outcome from hESCs is poorly characterized in the scientific literature and is a source of concern (Siniscalco, et al, 2013)

As this data relates to ASDs, it has been demonstrated that an altered immune cell ratio is sometimes associated with a decreased number of T lymphocytes (Siniscalco, Sapone, et al, 2012). The ability of ESCs to differentiate into hematopoietic cell lineages, giving rise to all blood cell types and subtypes of the immune system (i.e.: T cells, NK cells, and dendritic cells), could be used in immune-altered pathologies, such as ASDs, which require induction of the immune response in an antigen-specific manner (Ng, Davis, et al, 2005; Nakamura, Hiroyama, et al, 2011; Senju, Hirata, et al, 2010). However, the complications encountered with the use of hESCs as a treatment modality outweigh the benefits and the long-term therapeutic benefits have proven to be suboptimal. As of the publication of this review, eight clinical trials were ongoing in the study of treating ASDs with the use of human stem cells however, none of these trials included the use of hESCs.

Based on these data, it is uncertain whether hESCs are ready for clinical use.

Fetal Stem Cells

Fetal Stem Cells (FSCs) can be isolated from fetal blood and bone marrow as well as from other fetal tissues (amniotic fluid, placenta, chorion, or umbilical cord). Those derived from fetal tissues are often referred to as extraembryonic FSCs due to their originating in the extraembryonic membranes. These FSCs are acquired after birth and are considered pluripotent. This pluripotency gives them the ability to differentiate into the three germ layers subtypes: ectoderm (including the brain), mesoderm, and endoderm. FSCs have great potential for clinical use; as they possess immune-regulatory functions found in mesenchymal stem cells yet they exhibit a greater expansion capacity and enhanced plasticity (Klemmt, Vafaizadeh, et al, 2011). Early gestational fetal neuronal tissue is of particular interest to neurodegenerative disease therapies and may serve as a model for ASD interventions. In part, this is because early FSCs have minimal or no expression of MHC-I and no MHC-II (Laguna Goya, Busch, et al, 2011). It has also been found that FSCs express HLA-G which belongs to the HLA class I heavy paralogues. HLA-G is involved in the presentation of foreign antigens to the immune system thereby providing a factor of tolerance. And, it is this added benefit that convenes increased viability post-transplantation.

Benefits that are seen in fetal mesenchymal stem cells that are not seen in ECSs are that they do not form teratoma post-transplantation and they are obtained from tissues that would otherwise be discarded as medical-waste. FSCs hold a multitude of additional benefits: they are known to exert strong immunomodulatory effects, they possess a stable phenotype, they demonstrate less senescence, they are able to release several diffusible neurotrophic and growth factors, and they have the capacity to suppress proinflammatory cytokines. An additional benefit of FSCs could be due to paracrine trophic actions on host tissues affected by ASDs, rather than cell replacement. Siniscalso and colleagues (2013) report that as FSCs are derived from all germ layers, they retain their tissue-specific instructions and are therefore regulated properly, unlike pluripotent ESCs. In this way, cell or tissue/organ FSCs could restore dysfunctional development of the brain, gut, and immune system.

The positive qualities and mechanism of action of FSCs may contribute to the success of allogeneic FSC transplants in ASD therapies.

Neural Stem Cells

Neural stem cells (NSCs) exist not only in the embryo, but also in the adult brain neurogenic region: the subventricular zone (SVZ) of the lateral ventricle. ESCs acquire NSC identity with a default mechanism. Under the regulations of leukemia inhibitory factor (LIF) and fibroblast growth factors, the NSCs then become neural progenitor cells (NPCs) – (Hsu, Lee, et al, 2007). It has been shown that these multipotent cells show self-renewing capacities and are able to generate multiple cell types of the mammalian central nervous system. The capacity of integration into neural tissue, replacing damaged cells and reconstructing neural circuitry are the main characteristics of NSCs with potential usefulness for treating ASDs. Research also shows that in autism, excitatory and inhibitory cortical neurons contribute to minicolumn structure abnormalities and functional imbalances in the cortex. It has been hypothesized that transplanted NPCs could promote neurogenesis through their contributing to the changes in the brain microenvironment. However, before being suitable for clinical applications in neurodegenerative diseases or ASDs, some critical issues with the use of NPCs require further investigation. A reliable source of sufficient autologous NPCs needs to be identified. Further, the regulation of postimplantation neural plasticity and differentiation of NSCs in the child or adult nervous system must be further defined (Hsu, Lee, et al, 2007).

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are defined as multipotent stromal cells of mesodermal origin which possess a self-renewing capacity. MSCs can be easily obtained and from multiple sources. These cells are found primarily in the bone marrow and adipose of children and adults but can also be derived from both the umbilical cord (UC), umbilical cord blood (UCB), and placenta. The cells acquired from bone marrow stroma and are typically obtained from donors via a bone aspirate surgical procedure through the iliac-crest, tibia, femur, or vertebrae while adipose cells are easily harvested, generally from the abdominal region, through a minimally-invasive small volume lipo-aspirate procedure. UC-MSCs and UCB-MSCs are both available in relatively large quantities from morally acceptable sources with collection consisting of no painful or invasive techniques.

Regardless of collection site, MSCs can differentiate into a multitude of cell types including osteoblasts, chondrocytes, myocytes, adipocytes, hepatocytes, cardiomyocytes, and neurons. This capacity of a cell to differentiate into mesenchymal lineages *in vitro* is one of the

essential requisites of the International Society of Cellular Therapy (ISCT) for a cell to be defined as an MSC. In regards to the overall research benefits of MSCs, the ISCT also reports that MSCs possess the capacity to grow in adherence to the plastic surface of dishes when maintained in standard culture conditions. When these standard culture conditions are maintained, MSCs can be readily stores and will quickly expand. The ISCT goes on to report additional minimal criteria for cells to be defined as MSCs: They must express cytospecific cell surface markers (CD105, CD90, and CD73) and must lack expression for all other surface markers (CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR).

Research has shown numerous benefits of MSCs, some of which include their immune-modulating capabilities and their absence of uncontrollable growth and tumor formation. These qualities attest to their clinical safety and to their usefulness for transplantation purposes and, upon transplantation, MSCs are able to readily migrate to the site of injury in order to assist in the restorative process. MSCs also possess strong anti-inflammatory and immunosuppressive activity, rendering them very attractive for successful autologous, as well as heterogeneous, transplantations without requiring pharmacological immunosuppression (Le Blanc & Pittenger, 2005; Petrie Aronin & Tuan, 2010). Additionally, since MSCs are able to express *in vivo* immunosuppressive factors, the need of genetic modification or pretreatment before transplantation are negated therefore, there is no further concern of immune rejection problems. It is this MSC-mediated immune system modulating activity that could prove to be a key mechanism in ASD therapy.

As previously discussed, immune-dysregulation is known to be one of the consistent findings with ASD diagnosis. ASD children show imbalances in CD3+, CD4+, and CD8+ T cells, as well as natural killer (NK) cells. In addition, peripheral blood mononuclear cells (PBMCs) extracted from ASD children show over production of caspase proteases, proinflammatory cytokines, and cannabinoid-type-2 receptors resulting in long-term immune alterations and proinflammatory cellular events (Siniscalco, Sapone, et al, 2012; Enstrom, Onore, et al, 2010; Siniscalco, Sapone, et al, 2013). A notable research consideration is that MSC immunoregulatory effects could restore the immune-dysregulation in ASD (Figure 3).

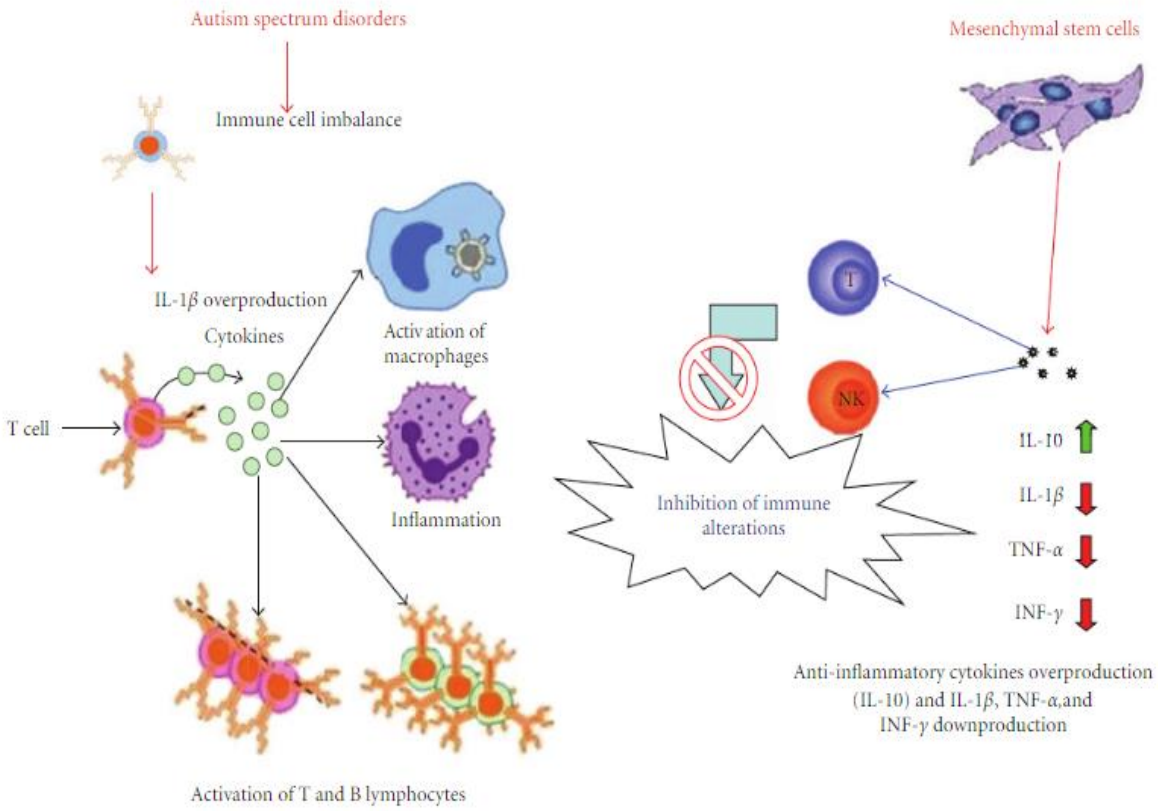

Figure 3. Paracrine and immunomodulatory effects as possible mechanisms of action of mesenchymal stem cells (MSCs) in autism spectrum disorder (ASD) treatment. In humans, ASDs are associated with immune alterations and pro-inflammatory cytokines (i.e., IL-1β) over-production. These cytokines are able to trigger pro-inflammatory cellular events. Data from in vitro models show that MSCs are able to affect not only T cells, but also other cells of the immune system (i.e., NK cells). Immunoregulatory properties of MSCs are through secretion of large amounts of several bioactive molecules (paracrine activity), that is, PGE-2, IL-10. These molecules cause the inhibition or the unresponsiveness of T-cell mediated responses. (Siniscalco, Sapone, et al. 2012).

MSCs could also have restorative effects on the neural-dysregulation of ASD. Supporting this potential application of cell therapies for ASDs, transplanted MSCs are able to promote synaptic plasticity and functional recovery (Rodrigues Hell, Silva Costa, et al, 2009; Chang, Chen, et al, 2011). Additionally, the mobilization of MSCs to the site of neural injury could provide potential ameliorative effects in ASD treatment (Table 2). This mobilization is a key facet in the proposed mechanism of action that MSCs have in regards to neural restoration and tissue repair.

Table 2

Potential ameliorative effects mediated by MSCs in ASD treatment

ASD-induced changes in human brain	Potential MSC ameliorative roles seen in preclinical models
Abnormal functioning	Improving functional recovery
Cerebellum alterations	Integrating in altered brain and restoring damaged functions
Decreased number of Purkinje cells (PCs)	Restoring cerebellar PCs
Defective cortical organization	Reinforcing cortical plasticity

Note. source: (Siniscalco, Sapone, et al. 2012).

MSCs have been proven to aid in the restorative factors associated with immune and neural dysregulation of ASDs, but there is now a burgeoning science dedicated to the role of epigenetic factors associated with ASDs and how MSCs can contribute to the amelioration of these factors. As we delineate, epigenetics is a mechanism that controls gene expression without changing DNA sequence but by changing chromosomal histone modifications and its abnormality is associated with several neurodevelopmental diseases. Since epigenetic modifications are known to be affected by environmental factors such as nutrition, drugs and mental stress, autistic diseases are not only caused by congenital genetic defects, but may also be caused by environmental factors via epigenetic mechanism (Miyake, Hirasawa, et al, 2012). Siniscalco and colleagues (2013) report that native MSCs in ASD could not inhibit the epigenetic processes triggered by the environmental factors which ultimately led to the development of the autistic phenotype.

Further research needs to be done in order to determine whether or not all autologous MSCs are capable of responding appropriately to induce the healing effects needed for neuro-rehabilitation.

Through this continued evaluation of each source of MSC we are reminded that MSCs can also be isolated from adipose tissue. Adipose tissue is derived from the mesoderm during embryonic development and is present in every mammalian species, located throughout the body. Adipose tissue serves as an endocrine organ, functioning to maintain energy metabolism

through the storage of lipids. While two types of adipose tissue exist (brown and white), white adipose yields the commonly studied adipose-derived mesenchymal stem cells (AD-MSCs) – (Minteer, Marra, et al, 2013). Research indicates that the clinical benefits of AD-MSCs are they exhibit anti-inflammatory characteristics, and have the ability to differentiate into other tissue types of the mesoderm-including adipogenic, osteogenic, chondrogenic, myocyte, and other mesenchymal lineage. Although clinical trials have been conducted using AD-MSCs in ASDs (Table 3) there is still much research to perform in order to determine their full clinical potential. Some such points to investigate are their differentiation processes into cell lineages apart from adipocytes post-reimplantation, Additionally, Siniscalco and colleagues (2013) report that there is no evidence that the re-administration of MSCs extracted from adipose tissue will overcome the intrinsic sense of the MSCs to return to the surgical harvest (lipo-aspirate) site to initiate repair. It is also likely that cell preparations from the lipo-aspirate might contain a heterogeneous population of cells which equates to concerns of cell purity and molecular phenotype.

Continued research on AD-MSC biology is needed before their use in ASD therapy can be further understood.

Table 3

Current Stem Cell Clinical Trials Associate with the Treatment of ASDs

Study	Condition	Intervention	Status	Link
Autologous Bone Marrow Stem Cells for Children with Autism Spectrum Disorders	Autism; Autism Spectrum	Other: Stem Cells	Recruiting	https://clinicaltrials.gov/ct2/show/NCT01740869?term=stem+cells+autism&rank=1
Autologous Bone Marrow Stem Cell Therapy for Autism	Autistic Disorder	Biological: Autologous Bone Marrow Mononuclear Cells	Completed	https://clinicaltrials.gov/ct2/show/NCT02627131?term=stem+cells+autism&rank=3
A Clinical Trial to Study the Safety and Efficacy of Bone Marrow Derived Autologous Cells for the Treatment of Autism	Autism	Biological: STEM CELL THERAPY	Unknown†	https://clinicaltrials.gov/ct2/show/NCT01836562?term=stem+cells+autism&rank=6
Stem Cell Therapy in Autism Spectrum Disorders	Autism Spectrum Disorders	Procedure: Autologous bone marrow mononuclear cell transplantation	Completed	https://clinicaltrials.gov/ct2/show/NCT01974973?term=stem+cells+autism&rank=7
Allogeneic Umbilical Cord Mesenchymal Stem Cell Therapy for Autism	Autism	Biological: Umbilical cord mesenchymal stem cells	Active, not recruiting	https://clinicaltrials.gov/ct2/show/NCT02192749?term=stem+cells+autism&rank=2
Safety and Efficacy of Stem Cell Therapy in Patients with Autism	Autism	Biological: human cord blood mononuclear cells; Biological: human cord blood mononuclear cells and human umbilical cord mesenchymal stem cells	Completed	https://clinicaltrials.gov/ct2/show/NCT01343511?term=stem+cells+autism&rank=4
Autologous Cord Blood Stem Cells for Autism	Autism	Biological: Autologous cord blood Stem Cells; Biological: Placebo	Active, not recruiting	https://clinicaltrials.gov/ct2/show/NCT01638819?term=stem+cells+autism&rank=5
Adipose Derived Stem Cell Therapy for Autism	Autism	Procedure: Fat Harvesting and Stem Cell Injection	Unknown†	https://clinicaltrials.gov/ct2/show/NCT01502488?term=stem+cells+autism&rank=8

Note. † = Study has passed its completion date and status has not been verified in more than two years.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are the prototypical MSC and are the most well studied of all stem cells. In 1999 Mark Pittenger and his colleagues first reported the multi-lineage potential of adult human mesenchymal stem cells. However, it is also reported that advanced donor age diminishes the proliferative capacity as well as having effects on the cells anti-inflammatory capacity and homing ability (Stenderup, Justesen, et al, 2003; Bustos, Huleihel, et al, 2014). This diminished cellular "fitness" marks BM-MSCs as a suboptimal choice when aiming to treat childhood disorders such as ASDs.

While BM-MSCs have been known to be the prototypical MSC for clinical purposes, there is emerging evidence that umbilical cord-derived and umbilical cord blood-derived mesenchymal stem cells (UC-MSCs and UCB-MSCs) might set a new 'platinum standard' of care. UC and UCB may also prove to be a richer source of MSCs, based on colony forming unit-Fibroblastic efficiency, and generate MSCs with greater immunomodulatory potential than BM-MSCs (Wegmeyer, Bröske, et al, 2013). Research continues to demonstrate that ASD, and its degrees of severity, have been significantly correlated with inflammatory and neuro-inflammatory cytokines including macrophage-derived chemokine (MDC) and thymus and activation-regulation chemokine (TARC) and based on these findings, it is hypothesized that umbilical cord-blood derived cell therapies may have the potential in alleviating ASD symptoms by modulating inflammatory processes in the brain (Riordan, 2016; Dawson, Sun, et al, 2017).

As the review of stem cells continues, data confirms that the continued advancement of stem cell research, as it pertains to treatments for ASDs, is more important now than it has ever been. According to the CDC, there has been a substantial increase in the reported cases of ASDs over the past several decades. Reports indicate that in 1996, 3.4:1000 children were diagnosed with ASDs as compared to their latest study (2012) indicating that 14.6:1000 children were diagnosed with ASDs. This is over a four-fold increase in only a 16 year time span. With this dramatic increase we are still struggling to understand the pathophysiologies associated with ASDs, the defined mechanisms of these pathophysiologies and most importantly: how we can render a curative therapy. Tremendous amounts of research has been conducted on the behavioral, nutritional, and pharmacologic treatments of ASDs however, there is still no optimized standard of care. Current research suggests that stem cell therapies represent the future of molecular and regenerative medicine for what would otherwise be untreatable human

diseases. Stem cells are also suitable for developing cell-based patient-specific pharmacotherapies (Siniscalco, Giordano, et al, 2012; Siniscalco, Pandolfi, et al, 2012). These characteristics suggest that stem cells can provide therapeutic applications and new treatment options for ASD patients. In order to develop these therapeutic applications, treatment modalities have had to endure years of stringent research and clinical trials before their clinical application is permissible.

Funding for such research is provided by a number of entities. The United States (U.S.) Governmental funding entity is that of the National Institutes of Health (NIH) which invests nearly $32.3 billion[1] annually in medical research for the American people. More than 80% of the NIH's funding is awarded through nearly 50,000 competitive grants to more than 300,000 researchers at more than 2,500 universities, medical schools, and other research institutions in every state and around the world (NIH, 2017). This budget is allocated to 265 research, condition, and disease categories - including human stem cell research and human stem cell research as it pertains to the treatment of ASDs. The NIH confirms that during the years of 2012 through 2017, an average of $916 million was granted to the area of general human-SC research (Table 4), and $11.5 million of those funds were then allocated to the subspecialty of studying the treatment of ASDs with the use of human stem cells (Table 5). These monies were allocated through 34 grants which were awarded to nine various research institutions (Table 6). There are two important caveats that should be taken into account when reading these data: These values do not include privately funded research projects nor do they include European trials/research.

[1] This amount reflects the sum of discretionary budget authority of $31,381 million received by NIH in FY 2016 under The Consolidated Appropriations Act of 2016, Public Law (P.L.) 114-113, $780 million derived from PHS Evaluation financing, and mandatory budget authority of $150 million for special type 1 diabetes research authorized per P.L. 113-93 and P.L. 114-10 (NIH, 2017).

Table 4

National Institutes of Health Universal Funding Rates for Human Stem Cell Research

SC Type Researched (dollars in millions & rounded)	Fiscal Year 2012 (actual)	2013 (actual)	2014 (actual)	2015 (actual)	2016	2017
					Estimated costs	
hESC	$146	$146	$166	$180	$190	$190
hiPSC	$175	$199	$280	$282	$296	$296
Non-ESC	$504	$431	$443	$445	$465	$465
UCB-SC & Placental SC	$43	$35	$28	$32	$32	$32
TOTAL FUNDING	$868	$811	$917	$939	$983	$983

Note. SC = Stem Cell; hESC = human Embryonic Stem Cell; hiPSC = human induced Pluripotent Stem Cell; ESC = Embryonic Stem Cell; UCB = Umbilical Cord Blood

Table 5

National Institutes of Health Funding Rates for Human Stem Cell Research devoted to ASD

	Fiscal Year			
SC Type Researched	2012	2013	2014	2015
hESC	$562,927	$594,332	$53,282	$56,042
hiPSC	$4,242,543	$2,477,840	$495,044	$882,480
Non-ESC	$1,384,929	$594,332	$87,500	$77,142
UCB-SC & Placental SC (listed under Non-ESC)	$0	$0	$0	$0
TOTAL FUNDING	$6,190,399	$3,666,504	$635,826	$1,015,664

Note. SC = Stem Cell; hESC = human Embryonic Stem Cell; hiPSC = human induced Pluripotent Stem Cell; ESC = Embryonic Stem Cell; UCB = Umbilical Cord Blood. There have been no estimated costs associated with Stem Cell research devoted to ASD for the years 2016 and 2017.

Table 6

Institutional Funding Rates from the NIH for Human Stem Cell Research devoted to ASD

Institution	Total amount funded (2012-2015)	Number of grants received (2012-2015)
Children's Hospital of Orange County, CA	$3,324,135	6
NIH, Md	$3,305,437	4
Stanford University, CA	$2,874,582	14
Hugo W. Moser Research Institute – Kennedy Krieger, Md	$619,801	1
Scripts Research Institute, CA	$460,152	1
Yale, CT	$393,455	1
University of California - Davis	$324,625	3
University of California – San Francisco	$120,904	1
University of California – Los Angeles	$85,318	3
TOTAL:	$11,508,409	34

Note. NIH = National Institutes of Health

A multitude of clinically relevant research findings have been the result of such European or privately funded trials.

An example of a key European trial was the 2014 open label, prospective, pilot-study conducted by EmCell (Ukraine) evaluating the efficacy of FSC transplantation in ASDs. This was a multi-center (nine country) study with a primary endpoint objective to assess and establish the safety of FSC transplantation (FSCT) in children with autism. The secondary endpoint objective was to evaluate the effectiveness of FSCT for reducing the core symptoms of ASDs (Bradstreet, Sych, et al, 2014). The study design shows that 45 subjects were chosen who had a confirmed diagnosis of autism (DSM-IV-TR criteria). The subject population consisted of 39 males and 6 females ranging in age from 3 to 15 years old (mean = 6.94±0.89). Subjects were monitored pre-implantation and then six and 12 months post-transplantations. Transplantations consisted of two doses of intravenously and subcutaneously administered FSCs. ASD tests were also performed on the subjects: The Autism Treatment Evaluation Checklist (ATEC) and the Aberrant Behavior Checklist (ABC). In addition, laboratory examinations and clinical assessments of adverse effects (AEs) were performed in order to evaluate treatment safety. The study findings resulted in no significant AEs observed in the treated subjects, including no transmitted infections or immunological complications. Statistically significant differences ($p<0.05$) were noted on both the ATEC and ABC scores for the domains of speech, sociability, sensory, and overall health, as well as reductions in the total scores when compared to pretreatment values (Bradstreet, Sych, et al, 2014). Although the results of this pilot-study are promising, further research is merited.

While FSCs show promise in allogeneic use, NPSs need further research before proof of efficacy in clinical application. Moreover, Human Mesenchymal Stem Cells (hMSCs) and their subtypes have been the subject of the most recent research papers and numerous clinical trials associated with the use of stem cells in the treatment of autism spectrum disorders. As of the date of this publication, a service of the NIH: clinicaltrials.gov, reported eight open clinical trials in the use of human Stem Cells (hSCs) for the treatment of ASDs (Table 2). Four of said trials utilized bone marrow mononuclear cells as their intervention source, three trials utilized umbilical cord SCs, and one trial utilized adipose tissue derived cells. Of these trials, all were privately funded and 7:8 were being conducted outside of the United States.

As research continues to advance in efforts to uncover the most efficient and effective stem cell treatment for ASDs, current studies are revealing that a number of U.S. research institutions are establishing themselves as leaders in the field of stem cell therapy for ASDs. The most prominent of these facilities is Duke University which has established the Duke Center for Autism and Brain Development. Researchers at this facility, in conjunction with the Duke University Medical Center, are conducting a groundbreaking program of research to evaluate the efficacy of autologous and allogeneic cord blood for improving outcomes of children with autism spectrum disorders.

Most recently (2017) Dr. Dawson and her colleagues at the Duke Stem Cell Transplant Laboratory published their findings related to their single-center phase I open label trial which demonstrated the safety and efficacy of autologous cord blood infusions to treat ASD. In reviewing the study design and methods we find that twenty-five participants (21 males, 4 females) were enrolled. These participants ranged in age from 2 to 5 years, with a median age of 4.62 years. The median nonverbal IQ equaled 65 (range 22-123). It was also reported that 72% of the participants had moderately severe or severe ASD symptoms. Inclusion measures for these participants was that they (a) must meet the clinical diagnostic criteria for an ASD diagnosis (per DSM-5 criteria), (b) must have a nonverbal intelligence quotient (IQ) of ≥ 35 on the Stanford-Binet Intelligence Scales for Early Childhood, Fifth Edition or Mullen Scales of Early Learning, (c) have availability of a qualified autologous umbilical cord blood unit (viable CD34+), (d) the participant must be stable on their current medications for at least 2 months prior to the infusion,

(d) they must have the ability to travel to Duke University three times (baseline and 6 and 12 months post-baseline), and (e) parents must be English speaking.

Exclusion criteria included (a) a history of prior cell therapy, (b) use of intravenous immunoglobulin or other anti-inflammatory medications (with the exception of NSAIDs), (c) known genetic (e.g., fragile X) or other significant medical comorbidity, (d) obvious physical dysmorphology suggestive of a genetic syndrome, (e) an uncontrolled seizure disorder, (f) significantly impaired renal or liver function, and (g) clinically significant abnormalities in complete blood count (Dawson, Sun, et al, 2017).

Procedurally, the subjects received a single intravenous infusion of autologous umbilical cord blood. On the day of infusion, the cord blood was thawed and washed in dextran 40 + 5% albumin (DA) and placed in 1.25 ml/kg DA for administration (Rubinstein, Dobrila, et al; 1995). Thawed cord blood units were tested for enumeration of total nucleated cell count (TNCC), viable CD34+ cells, colony-forming units (CFUs), cell viability via trypan blue, and sterility cultures. The autologous umbilical cord blood infusion was performed following a sedated brain magnetic resonance imaging scan (MRI). IV access was obtained by a pediatric anesthesiologist. When the MRI was complete, children were admitted to the Duke Children's Health Center Day Hospital, an outpatient treatment center, for their infusion. After premedication with Benadryl (0.5 mg/kg IV), Solu-Medrol (0.5 mg/kg IV), and, if the child was awake and able to take oral medications, Tylenol (10 mg/kg PO), participants received either a portion of or their entire cord blood unit, adjusted to deliver $1–5 \times 10^7$ cells per kilogram, via peripheral IV infusion over 2 to 30 minutes. Intravenous fluids were administered at 1.5 times maintenance for 30 minutes to 2 hours after the cord blood infusion. (Dawson, Sun, et al, 2017).

The primary endpoint of this trial was to evaluate the safety of autologous cord blood infusions in ASD patients. Infusion reactions were monitored during time of procedure and additional AEs were identified through phone interviews with participants' parent/guardian at 7-10 days, 3 months, and 9 months after infusion. Follow-ups were also made in person at baseline, and at 6- and 12-month clinic visits. All reported AEs were graded as Mild (71 events) or Moderate (21 events). No serious AEs were reported. 13% of the AEs were considered related to the infusion, with the most common being allergic reaction, manifested by urticartia and/or cough occurring on the day of infusion (5 events in 4 participants; all Mild; 2 requiring an

additional dose of IV Benadryl). The most common unrelated AEs were agitation, skin changes, and typical childhood infections, reported between 2 days and 1 year post-infusion. There were no infusion-related infections or bloodstream or serious infections noted in any patient (Dawson, Sun, et al, 2017). Dawson and her colleagues reported that the assessment of AEs over the 12-months post-infusion indicated that cord blood infusions were safe and well tolerated.

Secondary clinical assessments were also carried out to determine both feasibility of administration and utility as an endpoint for potential phase II and III clinical trials. The assessments included the Vineland Adaptive Behavior Scales-II (VABS-II), Clinical Global Impression Scale (CGI), Pervasive Developmental Disorder Behavior Inventory (PDDBI), Expressive One-Word Picture Vocabulary Test-4 (EOWPVT-4), Behavior Assessment for Children-Social Skills subscale, Aberrant Behavior Checklist, Sensory Experiences Questionnaire, Repetitive Behavior Scale, Intelligence Scales (Mullen Scales of Early Learning or Stanford-Binet), Language Environment Analysis, Preschool Age Psychiatric Assessment, ATN GI Symptoms Inventory, and Parenting Stress Index. In addition, three objective biomarkers were collected: Eye Gaze Tracking of Social Stimuli (EGT), EEG, and brain MRI (Dawson, Sun, et al, 2017). Dawson and her colleagues reported that significant improvements in behavior were found across a wide range of outcome measures including: improvements in parent-reported measures including the VABS-II Socialization, Communication, and Adaptive Behavior Scores and the PDDBI, clinician assessments including the CGI-S, CGI-I, and EOWPVT, and objective eye gaze tracking measurements. Most of the observed behavioral changes occurred during the first 6 months and were sustained between 6 and 12 months post-infusion. A robust finding was that children's nonverbal IQ was correlated with change for the majority of outcomes measures, with higher nonverbal IQ being associated with greater improvements in behavior (Dawson, Sun, et al, 2017).

The results of this study were robust enough that Dawson and her colleagues are now using these data to focus on a second study to determine the efficacy of umbilical cord blood infusions in children with ASD (DukeACT). This study tests the best available donor, which will include an allogeneic group in addition to autologous and placebo groups.

DukeACT is a Phase II, interventional, parallel group, single site, prospective, randomized, double-blind study of a single intravenous autologous or allogeneic [unrelated cord blood (CB)] infusion in children ages 2-7 years with Autism Spectrum Disorder (ASD). DukeACT anticipates their sample population to exceed 160 subjects to be followed for ≥12 months. Participants will be randomly assigned to Sequence A, consisting of a single infusion of CB cells at baseline followed 6 months later by a single infusion of placebo, or Sequence B, consisting of an infusion of placebo at baseline followed 6 months later by an infusion of CB cells. All participants will ultimately be treated with CB cells at some point during the study. Participants with an available qualified autologous CB unit will receive autologous cells, and those without a suitable autologous CB unit available will receive cells from a ≥4/6 HLA-matched, ABO-matched allogeneic, unrelated donor CB unit from the Carolinas Cord Blood Bank. All infusions will be double-blinded. The primary outcomes will be assessed 6 months after the initial infusion in the sequence. Additional testing for secondary exploratory analyses will be performed at 12 months. Duration of study participation will be 12 months from the time of baseline infusion. (ClinicalTrials.gov Identifier: NCT02847182).

The significance of this study is multifold. Duke researchers have addressed the fact that not all ASD patients and their families have access to autologous SC's thus they have expanded their study to include allogeneic treatment options. Secondly, the Duke Research team has created an exhaustive list of primary and secondary measures to be evaluated. And lastly, a suitable inclusion/exclusion criteria has been established.

The primary endpoint of the DukeACT study is the change in social communication skills (a core symptom of autism) from baseline to six months after the initial study infusion, as measured by the Vineland Adaptive Behavior Scale (VABS)-II Survey Interview Form, Socializations Subscale Standard Score. The Control (placebo) and treated patients will be compared. Whereas the secondary outcome measures to be evaluated include: Change in Vineland Socialization domain raw score and domain age equivalent (Time Frame: Baseline, 6 months), change in Pervasive Developmental Disorder Behavior Inventory (PDD-BI) composite standard score (parent questionnaire) [Time Frame: Baseline, 6 months], Change in CGI-S and CGI-I (clinician assessment) [Time Frame: Baseline, 6 months], Change in Expressive One-Word Picture Vocabulary Test (clinician assessment) [Time Frame: Baseline, 6 months],

Change in Vineland Adaptive Behavior Communication subscale standard score, Daily Living subscale standard score, and Adaptive Behavior Composite [Time Frame: Baseline, 6 months], Change in individual subscales of the PDD-BI t scores [Time Frame: Baseline, 6 months], Incidence of infusion reactions [Time Frame: 12 months], Severity of infusion reactions [Time Frame: 12 months], Grade/severity will be assessed according to CTCAE v4.0 guidelines, Incidence of product-related infections [Time Frame: 12 months], Severity of product-related infections [Time Frame: 12 months], Grade/severity will be assessed according to CTCAE v4.0 guidelines, Evidence of alloimmunization via anti-HLA and anti-RBC antibodies and nonspecific markers of systemic inflammation (ESR, CRP) [Time Frame: 12 months], Incidence of graft vs. host disease [Time Frame: 12 months], Severity of graft vs. host disease [Time Frame: 12 months], Grade/severity will be assessed according to CTCAE v4.0 guidelines, Incidence of unexpected adverse events, by relation to study product [Time Frame: 12 months], Severity of unexpected adverse events, by relation to study product [Time Frame: 12 months], and Grade/severity will be assessed according to CTCAE v4.0 guidelines.

The inclusion/exclusion criteria indicate that both male and female subjects are accepted if their age is ≥ 2 years to ≤ 7 years (7 years, 364 days) at the time of visit 1 and if they have a confirmed clinical DSM-5 diagnosis of Autism Spectrum Disorder using the DSM-5 Checklist. The subjects must also have Fragile X testing performed and show a negative result. Subjects must also have available and qualified umbilical cord blood unit with a minimum banked total nucleated cell dose of ≥ 2.5 x 107 cells/kg that meets criteria outlined in their protocol which includes either autologous umbilical cord blood unit or ≥4/6 HLA-matched and ABO/Rh-matched allogeneic unrelated umbilical cord blood unit from the Carolinas Cord Blood Bank. Subjects must also be stable on current psychiatric medication regimen (dose and dosing schedule) for at least 2 months prior to infusion of study product. They must have a normal absolute lymphocyte count (≥1500/uL). The participant and parent/guardian are English speaking and be able to travel to Duke University two times (baseline and 6 months post-baseline), and parent/guardian must provide parental consent and be able to participate in interim surveys and interview.

Subjects are also evaluated very stringently for exclusion criteria. General exclusion criteria include: If a review of medical records indicates ASD diagnosis not likely, if there is a

known diagnosis of any of the following coexisting psychiatric conditions: depression, bipolar disorder, schizophrenia, obsessive compulsive disorder, Tourette syndrome. If screening data suggests that participant would not be able to comply with the requirements of the study procedures, including study outcome measures, as assessed by the study team, they will be excluded. If the family is unwilling or unable to commit to participation in all study-related assessments, including follow up for approximately 12 months, the subject will be excluded. And, if a sibling is enrolled in this study (DukeACT), a second is not allowed to enroll.

In regards to genetic and infectious exclusion criteria, Duke states the following: If 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 known pathogenic copy number variation (CNV) associated with ASD (e.g., 16p11.2, 15q13.2, 2q13.3) they will be excluded. If the subject has a known active central nervous system infection, has evidence of uncontrolled infection based on records or clinical assessment, or is HIV positive, the subject will be excluded.

General medical exclusion criteria include: known metabolic disorder, known mitochondrial dysfunction, history of unstable epilepsy or uncontrolled seizure disorder, infantile spasms, Lennox Gastaut syndrome, Dravet syndrome, or other similar chronic seizure disorder, active malignancy or prior malignancy that was treated with chemotherapy, history of a primary immunodeficiency disorder, history of autoimmune cytopenias (i.e., ITP, AIHA), coexisting medical condition that would place the child at increased risk for complications of sedation or other study procedures, concurrent genetic or acquired disease or comorbidity(ies) that could require a future stem cell transplant, significant sensory (e.g., blindness, deafness, uncorrected hearing impairment) or motor (e.g., cerebral palsy) impairment, 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, significant hematologic abnormalities defined as: Hemoglobin <10.0 g/dL, White blood count < 3,000 cells/mL, absolute lymphocyte count <1000/uL, Platelets <150 x 10e9/uL, or evidence of clinically relevant physical dysmorphology indicative of a genetic syndrome as assessed by the PIs or other investigators, including a medical geneticist or psychiatrists trained in identifying dysmorphic features associated with neurodevelopmental conditions (Kurtzberg, J., 2016).

The hope of such studies is to establish efficacy, safety, and tolerability parameters which will allow therapeutic access to all children affected with ASD rather than only to those who have access to banked autologous cord blood and, it is hopeful that research such as the DukeACT trial will continue to spawn additional studies to promote the advancement of stem cell treatments for ASD and other neurodevelopmental disorders.

In an additional review written by Dr. Ichim and his colleagues (2007), they also studied the therapeutic benefits of cord blood CD34+ cells (autologous and allogeneic) however, they proposed the combined use of CD34+ cord blood cells with MSCs in the treatment of ASDs. It is believed that the combining of MSCs with CD34+ induces synergistic effects in neurological diseases. Ichim discusses the fact that MSCs have been proven to suppress pathological immune responses while also stimulating hematopoiesis which leads to the possibility that these cells may be useful for treatment of the defect in T cell numbers associated with autism (Ichim, Solano, et al; 2007). Several studies have also confirmed that systemic administration of cord blood cells is sufficient to induce neuroregeneration (Newman, Willing, et al, 2006; Chen, Chang, et al, 2006; Peterson, 2004). And, given the potency of cord blood CD34+ cells to induce angiogenesis in areas of cerebral hypoperfusion, it is proposed that this cell type would be particularly useful in the treatment of ASDs.

Taking into account all sources of research data, to date, the most prolific and promising research published on the mesenchymal stem cell subtypes has been focused on the use of CD34+ umbilical cord cells however, we must recognize that further research is needed to understand these cells true mechanism of actions and their ability to act as 'biophamacies' capable of manufacturing a full array of cell-signaling chemistries.

Discussion

As of 2017 there has been a significant break from U.S. medical dogma stating that adult stem cell therapies are unproven and that only embryonic stem cells should be considered for therapeutic study. Duke University signaled its embrace of autologous stem cell therapy through their pivotal trials conducted at the Duke Stem Cell Transplant Laboratory, in conjunction with the Duke University Medical Center [Phase I & Phase II (DukeACT)].

Research and history have taught us lessons about stem cell research and its association to treating ASDs. We have learned that ESC research has all but ceased not merely due to their overwhelming ethical and physiological burdens but more importantly, due to their numerous failed trials. We have come to understand that a reliable source of sufficient autologous NPCs needs to be identified therefore verifying that further research needs to be conducted before proof of efficacy can be demonstrated. We understand that the positive qualities and mechanism of action of FSCs may contribute to the success of allogeneic FSC transplants in ASD therapies. And, we have learned that most proliferative research has focused on MSCs and their subtypes due to their strong anti-inflammatory and immunosuppressive activities which render them very attractive for both autologous and allogeneic transplantations without requiring pharmacological immunosuppression. In review, we are reminded that research confirms that these implanted stem cells (whether autologous or donor) are able to affect the recipient immune system through two proposed mechanisms: (1) cell-to-cell contact activation mechanism, through which transplanted stem cells switch proinflammatory macrophages to anti-inflammatory macrophages (Zheng, Ge, et al, 2013; Siniscalco, Giordano, et al, 2011), and (2) the paracrine-secretome activity (Zemel'ko, Kozhukharova, et al, 2013).

Research also confirms that the biomedical facet of ASD treatment can be met with the burgeoning field of stem cell therapies. As of 2017, there are eight clinical trials registered with the United States National Institutes of Health (clinicaltrials.gov) all of which focused on a subtype of MSCs (Table 2). In addition to these registered trials, teaching institutions and private research facilities continue to offer ASD patients the opportunity to take part in their privately funded ASD treatment trials and treatments.

The United States was the first to achieve the initial milestones in stem cell *research* and continues with cutting edge research protocols (e.g.: Duke trials) however, from a broad clinical scope we have learned that the United States is not currently recognized as offering the most advanced *treatment* options available for ASD treatments. The top ASD Stem Cell Therapy Treatment Centers can be found in numerous countries around the world. Pertinent to the stem cells discussed in this review, a selection of these ASD stem cell treatment centers are summarized in Tables 7-11. These centers offer promising ASD stem cell therapies which

include autologous and allogeneic options from a variety of stem cell types which were examined in this review.

Table 7 depicts data on the *Stem cell Institute* which is located in Panama. The *Stem Cell Institute* utilizes a five-day protocol of four intravenous infusions of human umbilical cord tissue-derived allogeneic mesenchymal stem cells. This facility is one of the most tenured in offering ASD stem cell therapy, having performed over 10,000 procedures from 2006 through 2017.

Table 7

Stem Cell Institute

Treatment Center	Stem Cell Institute
Location(s)	•Panama
Source of Cells	•Umbilical cord tissue-derived mesenchymal stem cells (allogeneic) •Umbilical cords are donated by mothers after normal, healthy births. Before they are approved for treatment all umbilical cord-derived stem cells are screened for viruses and bacteria to International Blood Bank Standards. In some cases, stem cells harvested from the patient's own bone marrow are utilized (autologous).
Number and Type of Treatments	•Protocol: -Treatment length (Monday – Friday): 5 Days -Physical examination and blood testing: Monday -4 intravenous infusions of human umbilical cord tissue-derived allogeneic mesenchymal stem cells: Tuesday – Friday •Administered intravenously by a licensed physician.
Cost	Dependent on type of treatment required
Insurance Coverage	None
Effectiveness of Treatment	•Performed over 10,000 procedures since 2006 but no efficacy rates provided
Contact Information	Web address: cellmedicine.com Address: BICSA FINANCIAL CENTER y Avenida, Calle Aquilino de la Guardia Panamá, Panama Aquilino de la Guardia Street BICSA Financial Center 63rd Floor Toll Free (US Only): 1-800-980-STEM (7836) From Outside or Inside US Call: 1-954-358-3382 Toll Free Fax (US Only): 1-866-755-3951 From Outside US Fax: 1-775-887-1194

Beike Biotechnology – Stem Cell Treatment Center, with locations in China and Thailand, is yet another example of a facility which offers umbilical cord blood and umbilical cord tissue ASD treatments (Table 8). Beike is a facility of importance due to its stringent treatment protocols which do not compromise on safety. These protocols have resulted in more than 22,500 patients receiving ASD stem cell treatments with no serious adverse reactions being reported.

Table 8

Beike Biotechnology – Stem Cell Treatment

Treatment Center	Beike Biotechnology
Location(s)	•Shenzhen, China •Bangkok, Thailand
Source of Cells	Umbilical cord blood and umbilical cord tissue
Number and Type of Treatments	•Stem cell treatment protocols require a stay of 16 to 40 days depending on the treatment protocol prescribed for the patient specific condition. In addition to multiple stem cell administrations, a comprehensive therapy program is being provided on a daily basis throughout the entire treatment. •Made through intravenous or intrathecal injections
Cost	Case dependent
Insurance Coverage	None
Effectiveness of Treatment	•Beike treatment protocols do not compromise on safety and adult stem cells have been used effectively for more than 22,500 patients with no serious adverse reaction reported. •ASD patients have experienced Improved social interaction better communication, improved speech, improved learning ability, decreased repetitive behavior, better mental development, improved bowel movement, and increased muscle tone
Contact Information	Web address: beikebiotech.com, stemcelltreatmentnow.com Tel: +86 755 8630 9200 Address: 16F Beike Building, 18 Keyuan Rd., South Area, Hi-Tech Industrial Park, Nanshan, Shenzhen, China 518057

Note. ASD = Adult Stem Cell.

The *Regeneration Center of Thailand* (Table 9) offers a 10 to 17 day protocol of enriched MSC injections for the treatment of ASD. This facility boasts an overall efficacy rate of 85% in over 2,500 patients treated (as of the year 2017).

Table 9

Regeneration center of Thailand

Treatment Center	Regeneration Center of Thailand
Location(s)	Bangkok, Thailand
Source of Cells	•Hematopoietic Mesenchymal Enriched Stem cells •The age and lack of physical development of a child may prohibit them from donating stem cells from adipose (fat) tissue or bone marrow or dental pulp derived stem cells. If the child is not a candidate for *Autologous* therapy or has severe conditions then *Allogeneic* HLA matched Mesenchymal stem cells from cord blood, amniotic membrane or placenta derived cells will be used after HLA matching is complete. •2 to 6 Enriched Mesenchymal Stem Cell Injections per treatment stage. •10 to 17 days •Multiple stages may be required for severe cases •Depending on the patients requirements the treatment injections will be made via a Guided CT Scanner (when necessary) or through a non-invasive and painless Intravenous Drip without anesthesia. Direct injections or Intrathecal injections may be required in some cases.
Cost	Case dependent
Insurance Coverage	None
Effectiveness of Treatment	Overall efficacy of treatments is rated at 85% in approximately 2,500 patients treated
Contact Information	Web address: stemcellthailand Address: 10110 Bangkok Klongton-Nue, 808/8 Thararom 2, Sukhumvit 55 FL 2, Thailand Tel: (+66) 808 069 391 Email: info@stemcellthailand.org

Note. HLA = Human Leukocyte Antigen; CT = Computed Tomography.

EmCell Treatment Center (Table 10) is a facility that is on the forefront of ASD-SC treatments and research, having conducted clinical trials evaluating the efficacy of FSC transplants in ASDs. EmCell has treated more than 300 autistic children to date (2017) with an overall success rate of 81%. Their data also reports that 91% of treatments resulted in increased attention, concentration, self-care, verbal skills, resolution of bowel problems, and overall functional independence.

Table 10

EmCell Treatment Center

Treatment Center	EmCell (Cell Therapy Center)
Location(s)	Ukraine
Source of Cells	•7–12 week old fetal stem cells harvested from legally aborted embryos and subjected to multiple safety testing. These cells preserve their pluripotent properties (ability to differentiate into cells of different germ layers: mesenchymal, ecto- and endodermal) and are the most effective in different diseases and conditions.
Number and Type of Treatments	•Average duration of FSC treatment course varies from 2 to 5 days and depends on the diagnosis, history of illness, complications or concomitant diseases. In order to inform the patient about the time needed for treatment, we require providing us with basic medical data. •Treatment is administered intravenously into the arm vein and/or subcutaneous injections are made in the frontal abdominal wall.
Cost	•Price of the treatment is defined by many factors such as: diagnosis, patient's condition, number of types of cells, which are used during the treatment. •The price of the treatment may vary from $68,000 to $80,000 USD
Insurance Coverage	None
Effectiveness of Treatment	•Treated >300 autistic children with success rate of 81% •91% of treatments resulted in increased attention, concentration, self-care, verbal skills, resolution of bowel problems, and overall functional independence.
Contact Information	Web address: emcell.com Tel.: +38 044 223 28 95 Fax: +38 044 468 80 29 Address: 37 A Syretska street, Kyiv 04073, UKRAINE

Note. FSC = Fetal Stem Cell; USD = United States Dollars.

A treatment center that incorporates gene therapies with their stem cell treatments for their ASD protocols is: *Stem Cell Genetic Med*, located in Wellington Florida (Table 11). *Stem*

Cell Genetic Med offers a unique protocol for their ASD patients. Potential patients are evaluated by checking for abnormal gene mutations or mitochondrial abnormalities. If an individual is found to be a candidate for their treatment, a one day protocol is established. The concept of their protocol is to rewire the brain allowing nerve cells to communicate through their axons not only locally but to distant areas of the brain.

Table 11

Stem Cell Genetic Med Treatment Center

Treatment Center	Stem Cell Genetic Med
Location(s)	Wellington, Florida
Source of Cells	Autologous SC's harvested from bone marrow or adipose tissue
Number and Type of Treatments	•One day protocol •Consists of a four step process: (1) harvesting SC's from patient, (2) separation of SC's, (3) activation of SC's – enriched with patients own blood plasma, (4) treatment via intravenous drip method. •Combination protocol: Treatment consists of a combination of stem cell and gene therapies. Patients are evaluated by checking for abnormal gene mutations or mitochondrial abnormalities. (1)When a gene mutation is discovered it is knocked out by a specific shRNA agent (2)This is followed by inserting the normal gene into the DNA of neural brain stem cells using an attenuated viral vector. (3)The next step is the administration of neural brain stem cells with the normal gene into the brain and central nervous system along with BDNF, NGF, NT, GDNF, CDGF, and VEGF by lumbar spinal tap. (4)Patients post treatment are given neuro brain stimulation externally with a device applied to the forehead and scalp twenty minutes a day. (5) Hyperbaric oxygen treatments The general concept is to rewire the brain allowing nerve cells to communicate through their axons not only locally but to distant areas of the brain.
Cost	Not indicated
Insurance Coverage	None
Effectiveness of Treatment	Not indicated
Contact Information	Web address: stemcellgeneticmed.com Address: 10111 Forest Hills Blvd Suite 255 Wellington, FL 33414 Main Tel: (561) 557-3358 Email: bfeinermanstemcells@gmail.com

Note. SC = Stem Cell; BDNF = Brain-Derived Neurotrophic Factor; NGF = Nerve Growth Factor; NT = Neurotrophins; GDNF = Glial Derived Neurotrophic Factor; CDGF = ciliary derived growth factor; VEGF = vascular endothelial growth factor

As of 2017, these data associated with research and outcomes shows promise for the future of stem cell treatments associated with autism spectrum disorders.

Conclusion

This review advances the knowledge in the field of stem cell therapies for autism spectrum disorders first by providing an up to date educational foundation of the stem cells used in the treatment of ASDs, the most current treatment and research options, as well as current outcomes and funding data. Secondly, this review was meant to promote a contemplative framework for current and future stem cell scientists in the field of ASD research which might stimulate future preclinical and clinical studies. This review also provides a comparison of current procedures and outcomes achievable internationally to that of the United States. And, lastly, the treatment that an ASD patient and their family might choose to receive simply due to reading this review, or the totality and magnitude of potential novel research conducted simply through reading this review, could be life changing and groundbreaking.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders: DSM -5*. Washington, D.C: American Psychological Association.

"Autism." *www.stemcellgeneticmed.com*. New Florida Marketing, 2017. Web. 2 May 2017.

"Autism treatment." *www.emcell.com*. Web. 2 May 2, 2017.

Baio, J. (2008). Prevalence of autism spectrum disorders – Autism and developmental disabilities monitoring network, 14 sites, United States. *MMWR Surveillance Summit*. 61(3), 1-19.

Bjorklund, G., Saad, K., et al. (2016). Immune dysfunction and neuroinflammation in autism spectrum disorder. *Acta Neurobiologiae Experimentalis (Wars)*. 76(4), 257-268.

Bradstreet, J.J., Smith, S., et al. (2010). Biomarker-guided interventions of clinically relevant conditions associated with autism spectrum disorders and attention deficit hyperactivity disorder. *Alternative Medicine Review*. 15(1), 15-32.

Bradstreet, J.J., Sych, N., et al. (2014). Efficacy of fetal stem cell transplantation in autism spectrum disorders: An open-labeled pilot study. *Cell Transplantation*. 23(1), S105-S112.

Bustos, M.L., Huleihel, L., et al. (2014). Aging mesenchymal stem cells fail to protect because of impaired migration and antiinflammatory response. *American Journal of Respiratory and Critical Care Medicine*. 189, 787–798.

Center for Disease Control and Prevention. (2016). *Autism Spectrum Disorders – Data & Statistics.* Retrieved from https://www.cdc.gov/ncbddd/autism/data.html

Chang, Y-K, Chen, M-H, et al. (2011). Mesenchymal stem cell transplantation ameliorates motor function deterioration of spinocerebellar ataxia by rescuing cerebellar Purkinje cells. *Journal of Biomedical Science*. (18)1, article 54.

Chen, S.H., Chang, F.M., et al. (2006) Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heatstroke in the rat. *Experimental Neurology*. (199)67-76.

Dawson, G., Sun, J, et al. (2017). Autologous Cord Blood Infusions Are Safe and Feasible in Young Children with Autism Spectrum Disorder: Results of a Single-Center Phase I Open-Label Trial. *Stem Cells Translational Medicine*. 6(5), 1332-1339. doi: 10.1002/sctm.16-0474. Epub 2017 Apr 5.

Drago, D., Cossetti, C., et al. (2013). The stem cell secretome and its role in brain repair. *Biochimie*. doi: 10.1016/j.biochi.2013.06.020. Epub 2013 Jul 1.

Duke Center for Brain Development and Autism. *Efficacy of Umbilical Cord Blood Infusion for Improving Outcomes of Children with Autism Spectrum Disorder: DukeACT*. http://autismcenter.duke.edu/research/efficacy-umbilical-cord-blood-infusion-improving-outcomes-children-autism-spectrum-disorder. Duke University and Health System, 2015. Web. 10 August 2017.

Enstrom, A.M., Onore, C.E., et al. (2010). Differential monocyte responses to TLR ligands in children with autism spectrum disorders. *Brain, Behavior, and Immunity*. 24(1), 64–71.

Hsu, Y.C., Lee, D.C., et al. (2007). Neural stem cells, neural progenitors, and neurotrophic factors. *Cell Transplantation*. 16(2), 133–150.

Ichim, T.E., Solano, F., et al. (2007). Stem cell therapy for autism. *Journal of Translational Medicine*. 5(30). doi: 10.1186/1479-5876-5-30.

Klemmt, P.A., Vafaizadeh, V., et al. (2011). The potential of amniotic fluid stem cells for cellular therapy and tissue engineering. *Expert Opinion on Biological Therapy*, 11(10), 1297–1314.

Kurtzberg, J. (2016). Cord blood infusions for children with autism spectrum disorders (DukeACT). https://clinicaltrials.gov/ct2/show/NCT02847182?term=duke+cordblood&rank=1. Retrieved July 13, 2017.

Laguna Goya, R., Busch, R., et al. (2011). Human fetal neural precursor cells can up-regulate MHC class I and class II expression and elicit CD4 and CD8 T cell proliferation. *Neurobiology of Disease*. 41(2), 407–414.

Le Blanc, K. & Pittenger, M.F. (2005). Mesenchymal stem cells: progress toward promise. *Cytotherapy*. 7(1), 36–45.

Minteer, D., Marra, K.G., et al. (2013). Adipose-derived mesenchymal stem cells: biology and potential applications. *Advances in Biochemical Engineering and Biotechnology*. (129):59-71. doi: 10.1007/10_2012_146.

Ming, X., Brimacombe, M., et al. (2008). Autism spectrum disorders: concurrent clinical disorders. *Journal of Child Neurology*. 23(1), 6-13.

Miyake, K., Hirasawa, T., et al. (2012). Epigenetics in autism and other neurodevelopmental diseases. *Advances in Experimental Medicine and Biology*. 724, 91–98.

Nakamura, Y., Hiroyama, T., et al. (2011). Red blood cell production from immortalized progenitor cell line. *International Journal of Hematology*. 3(1), 5–9.

National Institute of Health. (2017). *Budget*. Retrieved from https://www.nih.gov/about-nih/what-we-do/budget.

Newman, M.B., Willing, A.E., et al. (2006).Cytokines produced by cultured human umbilical cord blood (HUCB) cells: implications for brain repair. *Experimental Neurology*. (199)201-208.

Ng, E.S., Davis, R.P., et al. (2005). Forced aggregation of defined numbers of human embryonic stem cells into embryoid bodies fosters robust, reproducible hematopoietic differentiation. *Blood*. 106(5), 1601–1603,

Noriega, D.B., & Savelkoul, H.F. (2014). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*. 173(1), 33-43. doi: 10.1007/s00431-013-2183-4

Peterson, D.A. (2004). Umbilical cord blood cells and brain stroke injury: bringing in fresh blood to address an old problem. *Journal of Clinical Investigation*. (114)312-314.

Petrie Aronin, C.E. & Tuan, R.S. (2010). Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells. *Birth Defects Research*. C90(1), 67–74.

Prokhorova, T.A., Harkness, L.M., et al. (2009). Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. *Stem Cells and Development*. 18(1), 47-54. doi: 10.1089/scd.2007.0266

Razavi, S., Razavi, M.R., et al. (2013). Comparing brain-derived neurotrophic factor and ciliary neurotrophic factor secretion of induced neurotrophic factor secreting cells from human adipose and bone marrow-derived stem cells. *Development, Growth & Differentiation*. 55(6), 648-655.

Riordan, N. (2016). Stem cell therapy – Autism. *Stem Cell Institute.* Retrieved from https://www.cellmedicine.com/stem-cell-therapy-for-autism/

Rodrigues Hell, R.C., Silva Costa, M.M., et al. (2009). Local injection of BDNF producing mesenchymal stem cells increases neuronal survival and synaptic stability following ventral root avulsion. *Neurobiology of Disease*. (33)2, 290–300.

Rubinstein, P., Dobrila, L., et al. (1995). Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proceedings of the National Academy of Sciences of the United States of* America. (92), 10119–10122.

Senju, S., Hirata, S., et al. (2010). Pluripotent stem cells as source of dendritic cells for immune therapy. *International Journal of Hematology*. 91(3), 392–400.

Stenderup, K., Justesen, J., et al. (2003). Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone*. 33, 919–926.

Siniscalco, D., Bradstreet, J.J., et al. (2013). Perspectives on the use of stem cells for autism treatment. *Stem cells International*. Article ID 262438. Retrieved from http://dx.doi.org/10.1155/2013/262438

Siniscalco, D., Giordano, A., et al. (2012). Novel insights in basic and applied stem cell therapy. *Journal of Cellular Physiology*. 227(5), 2283-2286.

Siniscalco, D., Giordano, A., et al. (2011). Long-lasting effects of human mesenchymal stem cell systemic administration on pain-like behaviors, cellular, and biomolecular modifications in neuropathic mice. *Frontiers in Integrative Neuroscience*. 5(79).

Siniscalco, D., Pandolfi, A., et al. (2012). State-of-the-art on basic and applied stem cell therapy; Stem Cell Research Italy-International Society for Cellular Therapy Europe, Joint Meeting, Montesilvano (PE)-Italy, June 10-12, 2011. *Stem Cell Development*. 21(5), 668-669.

Siniscalco, D., Sapone, A., et al. (2012). Autism spectrum disorders: is mesenchymal stem cell personalized therapy the future? *Journal of Biomedicine and Biotechnology*. Volume 2012, Article ID 480289. doi: 10.1155/2012/480289.

Siniscalco, D., Sapone, A., et al. (2013). Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. *Journal of Autism and Developmental Disorders*. 43(11), 2686-2695.

Siniscalco, D., Sapone, A., et al. (2012). The expression of caspases is enhanced in peripheral blood mononuclear cells of autism spectrum disorder patients. *Journal of Autism and Developmental Disorders*. 42(7), 1403-1410.

"Stem Cell of America - The future is already here." *www.stemcellofamerica.com*. Stem Cell of America, 2014. Web. 2 May 2017.

"Stem Cell Therapy – Autism." *www.cellmedicine.com*. Genesis Framework, 2016. Web. 2 May 2017.

"Stem Cell Treatment for Autism / ASD." *www.stemcelltreatmentnow.com*. Beike Biotechnology, 2017. Web. 2 May 2017.

Tang, C., Weissman, I.L., et al. (2013). Immunogenicity of *in vitro* maintained and matured populations: potential barriers to engraftment of human *pluripotent* stem cell derivatives. *Methods in Molecular Biology*. (1029), 17-31.

"Treatment for Autism Spectrum Disorders ASD." *www.stemcellthailand.org* . Regen Center, 2017. Web. 2 May 2017.

Wegmeyer, H., Bröske, A.M., et al. (2013). Mesenchymal stromal cell characteristics vary depending on their origin. *Stem Cells and Development*. 22, 2606–2618.

Zheng, G.P., Ge, M.H., et al. (2013). Mesenchymal stem cells in the treatment of pediatric diseases. *World Journal of Pediatrics*. 9(3), 197-211.

Zemel'ko, V.I., Kozhukharova, I.B., et al. (2013). Neurogenic potential of human mesenchymal stem cells isolated from bone marrow, adipose tissue and endometrium: a comparative study. *Tsitologiia*. 55(2), 101-110.