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Efficacy and risks in the use of human stem cells in the treatment of children with cerebral palsy

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EFFICACY AND RISKS IN THE USE OF HUMAN STEM CELLS IN THE TREATMENT OF CHILDREN WITH CEREBRAL PALSY

by

TAYLOR JAZRAWI

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Approved by

First Reader

Jonathan Wisco Ph.D.
Associate Professor, Anatomy & Neurobiology

Second Reader

Michael Sussman, M.D.
Honorary Medical Staff
Shriners Hospital for Children- Portland

Third Reader

Maryann MacNeil, M.A.
Assistant Professor, Anatomy & Neurobiology

DEDICATION

I would like to dedicate this thesis to my parents, Adele and Laith, who show me fulfillment that comes with a career in medicine and who encourage me to stay on course.

I would also like to dedicate this thesis to the Women (& Men) at the Initiative for Women with Disabilities at NYU Langone Orthopedic Hospital with whom I have happily shared the last five years with as a volunteer. You have inspired me to pursue a career as a physician. I will be a better, future care provider because of you. Specifically, thank you for this experience, Connie Lam.

Last, but not least, I would like to dedicate this thesis to Boston University School of Health, where I found my academic voice and first learned the power in investing effort into prevention resulting in sustainable impact.

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EFFICACY AND RISKS IN THE USE OF HUMAN STEM CELLS IN THE TREATMENT OF CHILDREN WITH CEREBRAL PALSY

JAZRAWI TAYLOR

ABSTRACT

Cerebral palsy (CP), depending on disease severity, can result in diminished quality of life not only from decreased function but from societal stigmatization. Coordinating various care provider appointments, expense of short-term treatments, difficulty navigating transportation, and relying on caregivers can complicate patient and family lives. The costs of CP are substantial on the healthcare system, with one managed Medicaid database averaged across 15 U.S states finding the average annual Medicaid costs for children with CP to be 15 times higher than children without CP and averaged to $22, 383 United States dollar (USD) compared to $1,358 USD respectively (Pulgar et al., 2019). Cost effective treatment and effective prevention strategies are increasingly warranted for the CP population.

Due to the varying manifestations associated with CP, a standardized treatment for this condition is challenging. Current treatments may enhance quality of life and temporarily reduce pain or discomfort, but they do not cure CP. While perinatal prevention strategies potentially provide the greatest chance to prevent CP from occurring and should be the focus of health care policy, financial barriers remain especially with strained health care budgets. While cure remains elusive, focus on treatments and prevention strategies to limit disease impact is paramount.

In the last decade some attention has turned to the use of stem cell treatments in children and adolescents with CP to provide more impactful outcomes with earlier intervention potentially limiting the devastating musculoskeletal effects seen with severe disease. While primarily results from clinical trials both nationally and internationally suggest stem cell treatment increases gross motor function in children and adolescents, questions remain whether these treatments provide clinically meaningful improvement compared to traditional therapies.

The goal of this thesis is to discuss the current pharmaceuticals and non-pharmaceutical treatments with rehabilitative therapies that are historically used to reduce severity of secondary manifestations associated with CP in children. Human stem cell clinical trials for CP will be reviewed to assess efficacy and risks as this treatment is translated into clinical practice for children. In addition to stem cell treatment, public health practices of prevention during prenatal visits will be reviewed as it is an encouraging method to reduce preterm births which are a risk factor for CP development.

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LIST OF ABBREVIATIONS

ADL	Activities of Daily Life
ADSC	Adipose Derived Stem Cells
BM-MSC	Bone Marrow Derived Mesenchymal Stem Cells
CFA	Comprehensive Functional Assessment
CP	Cerebral Palsy
GABA	Gamma-aminobutyric Acid
GMA	General Movement Assessment
GMFCS	Gross Motor Function Classification System
GMFM	Gross Motor Function Measure
HINE	Hammersmith Infant Neurological examination
hUC-MSC	Human Umbilical Cord Mesenchymal Stem Cells
ICF	International Classification of Functioning, Disability and Health
MRI	Magnetic Resonance Imagining
MSC	Mesenchymal stem cells
PD-MSC	Placental Derived Mesenchymal Stem Cells
PDMS-2	Peabody Developmental Motor Scales Second Edition
SEMLS	Single-event multilevel surgery
SDR	Selective Dorsal Rhizotomy
UCB	Umbilical cord blood
WHO	World Health Organization

GLOSSARY

Athetoid …….A form of dyskinetic CP resulting in slow, involuntary, and twitching movements

Dystonia ……...A form of dyskinetic CP resulting in involuntary and repetitive muscle contractions

Gait .....A person's pattern of walking

Intrathecal administration .....injection into the spinal canal

Jaundice ……Medical condition that presents as yellowing of the skin or whites from the eyes resulting from excess pigment bilirubin released from older red blood cells

Kernicterus...A more service form jaundice that is associated with brain damaged caused by high levels of bilirubin

Perinatal .....Relating to the time, usually several weeks before and after birth

Periventricular leukomalacia .....A type of injury that affects premature infants and classified as a softening of white brain tissue near the ventricles that house the cerebrospinal fluid (CSF)

Prenatal .....Relating to the time, usually before birth, during or relating to pregnancy

Rigid .....stiff muscles, resulting in difficulty in movements

Spastic …….explains velocity related tone, which is the increasing resistance to stretch during movement

Wharton's Jelly…gelatinous substance within the umbilical cord that contains stem cells

CHAPTER ONE

Etiology

CP is a neurological, non-progressive condition with varying etiologies occurring from pre-conception, prenatal and perinatal insults to both the mother and developing fetus (Sadowska et al., 2020). Although there are various definitions of cerebral palsy, the journal of Developmental Medicine and Child Neurology has defined cerebral palsy (CP) "as a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain…" (Rosenbaum et al., 2007). CP is also defined by motor impairments that influence development of secondary musculoskeletal conditions and a variety of health concerns (Rosenbaum et al., 2007). Historical CP was believed to be a result of ischemic conditions in the fetal brain (Sadowska et al., 2020). However, a variety of insults during these critical periods increases the risk of harmful environments that lead to CP development (Sadowska et al., 2020). The current understanding of risk factors associated with CP are shown in **Figure 1**.

Some factors that increase the risk of CP include fetal hypoxic ischemic encephalopathy, periventricular leukomalacia, multiple gestations, jaundice that may lead to kernicterus, infections (chorioamnionitis), certain medical conditions of the mother, any fetal growth restrictions, genetic mutations, and birth complications relating to placental detachment and uterine rupture (Center for Disease Control and Prevention [CDC], 2021). However, some causes of CP are unknown and make it challenging to treat (Center for Disease Control and Prevention [CDC], 2021). According to Novak et

al., the Report of the Australian Cerebral Palsy Register: birth years 1995-2012 found that prematurity of less than 37 weeks' gestation constitutes 43% of cerebral palsy cases documented in Australia (Novak et al., 2020). A 2013 metanalysis that reviewed CP prevalence between 1985-2011 found a pooled global prevalence of 2.11 per 1000 live births (Oskoui et al., 2013). Another metanalysis in Germany found a significant decrease in CP prevalence when gestational age increased (Himpens et al., 2008).

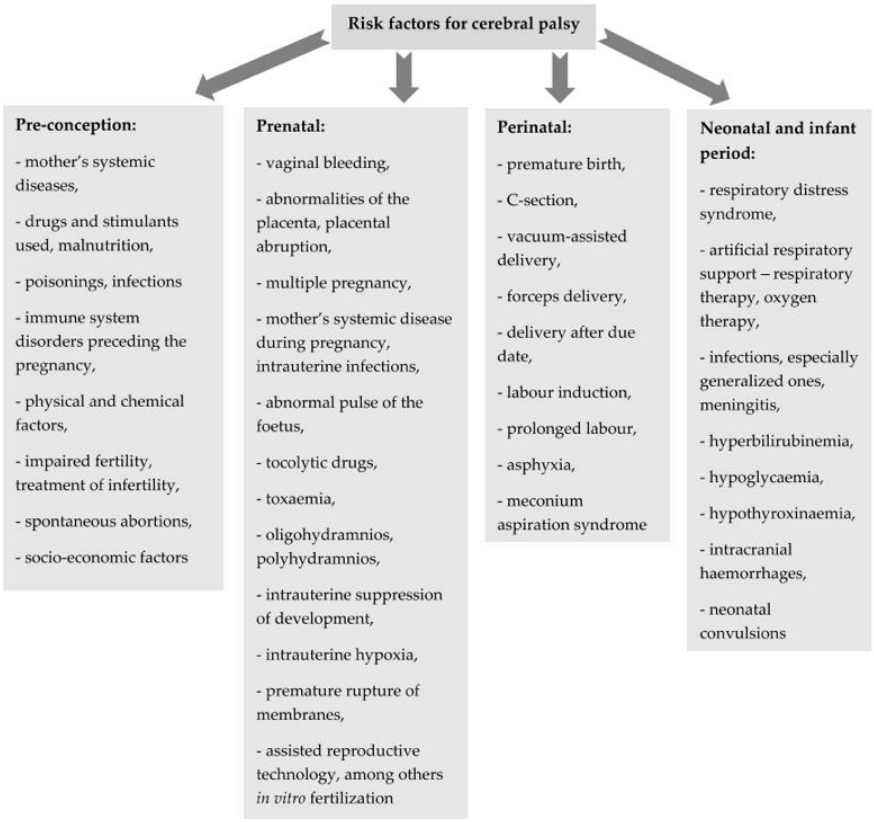


Figure 1: Risk Factors associated with CP. The figure suggests that irreversible brain damage to the fetus can occur during the pre-conception, prenatal, perinatal, and postnatal periods. Postanal insult can also result from physical trauma to the developing brain. These risk factors are important when thinking about future methods that potentially prevent CP. (Taken from Sadowska et al., 2020)

Clinical Classifications

Various types of CP include spastic, dyskinetic, or ataxic, which differ in motor abnormalities and rehabilitation options (Barn-on et al., 2015). The brain regions that give rise to the various motor types in CP are shown in **Figure 2**.

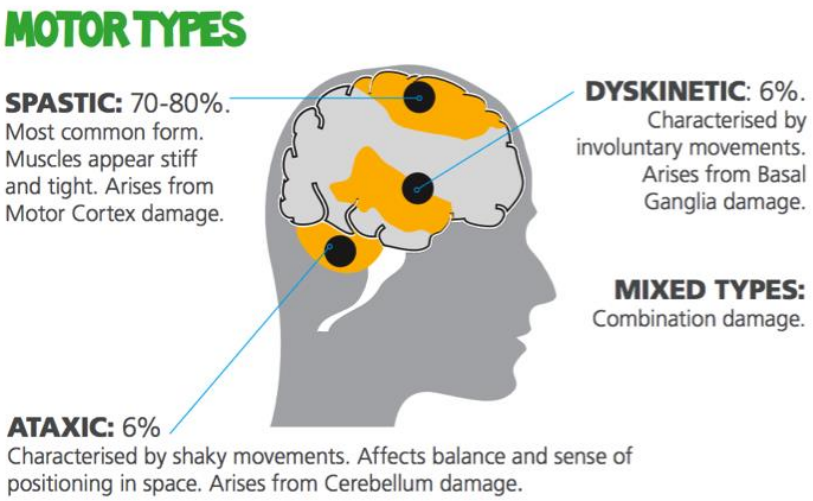


Figure 2: Various motor types of CP. Variations in brain region damage creates a diverse spectrum of physical limitations. The motor cortex controls voluntary movement. The basal ganglia region controls voluntary movements and orchestrates learned behaviors. The cerebellum controls balance and coordinated movement. Taken from (Cerebral palsy Scotland-The main types of cerebral palsy, n.d.).

Spasticity in CP results from diminished central nervous system inhibitory signals that act on the spinal cord reflex arc (Jea et al., 2019). The loss of inhibition on the reflex arc leads to continuous muscle contraction (Jea et al., 2019). According to Graham et al., spastic motor types are a common motor impairment observed with CP and a result of impaired neural circuitry within the pyramidal system or corticospinal tract region that project from the brain (Patel et al., 2020, Graham et al., 2016). Originally noted in Mukherjee et al., the pyramidal system is made up of the upper motor neurons, motor

cortex and runs from the cerebral cortex and brain stem, sending signals about movement to the spinal cord which house the lower motor neurons (Barn-on et al., 2015, Zayia & Tadi, 2021, Mukherjee et al., 2010). The topographical regions of the body that are affected by both pyramidal and extrapyramidal circuit impairments, involved in dyskinetic motor forms of CP, are presented in **Figure 3.** The lower motor neurons send signals directly to specific muscles to produce movement (Zayia & Tadi, 2021).

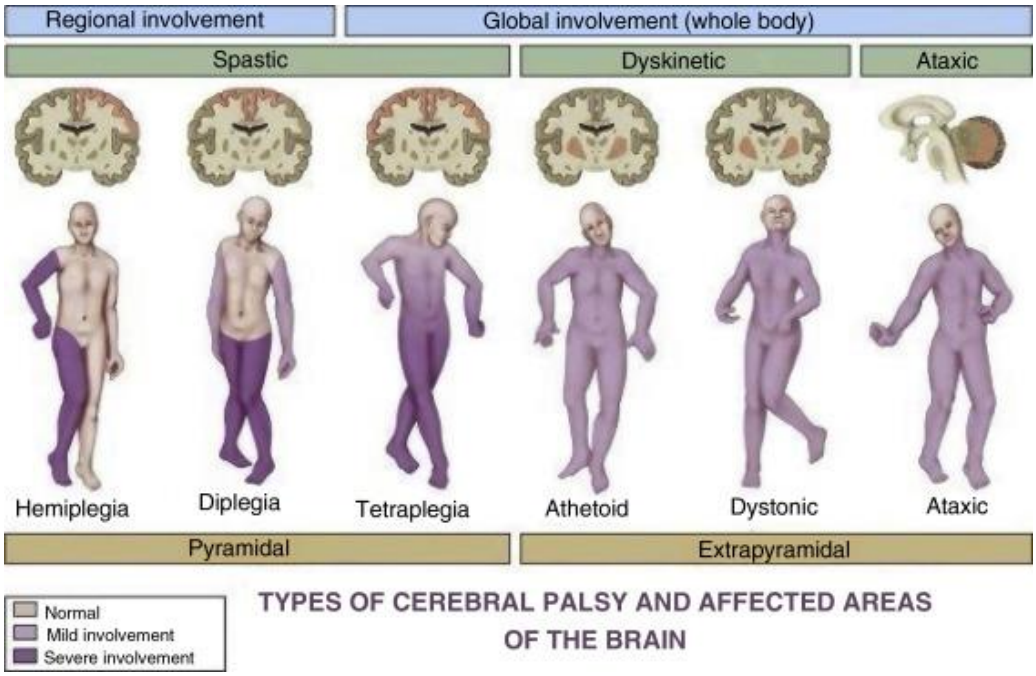


Figure 3: Cerebral regions with oxygen loss correspond to differences CP motor type. The pyramidal and extrapyramidal regions within the brain and brain stem respectively house motor neurons that descend into the spinal cord. Different motor types of CP are influenced by the brain region that sustained direct or unknown injury. Taken from (Cantero et al., 2021).

The spastic motor types are further differentiated into topographic regions that include spastic hemiplegia, diplegia, and quadriplegia CP and is often how CP is classified clinically (Patel et al., 2020, Velde et al., 2019). According to Bar-on et al., spasticity is associated with abnormality in tone known as hypertonia (Barn-on et al.,

2015). Originally noted by Sanger et al., hypertonia refers to resistance to passive motion, caused by stiff, spastic muscles (Barn-on et al., 2015). Clinical presentation of spastic hemiplegia indicates one side of the body is affected by CP whereas spastic diplegia primarily impacts both lower limbs (Velde et al., 2019). A severe spastic type comprised of functional limitations, cognitive deficiency and secondary manifestations of CP is spastic quadriplegia (Patel et al., 2020). Despite well documented prevalence of spastic CP in higher income countries, knowledge of prevalence and causes CP in lower- and middle-income countries remains limited, potentially underestimating global prevalence of CP (Kakooza-Mwesige et al., 2017).

Although organizing the brain lesions into the pyramidal and extrapyramidal regions may facilitate ease of anatomical understanding these definitions are now considered outdated (Lanciego et al., 2012). Damage within the extrapyramidal system of the brain is associated with dyskinetic forms of CP (Monbaliu et al., 2017). The extrapyramidal system contains the basal ganglia and subcortical nuclei, primarily involved in motor control and learning (Lanciego et al., 2012). According to the Surveillance of Cerebral Palsy in Europe, signs of dyskinetic CP are involuntary, uncontrolled, and repetitive movements (Cans et al., 2007). Ataxic CP is characterized by impaired motor coordination and balance (Sadowska et al., 2020, Cans et al., 2007).

Standardized clinical tools to access impact of CP

An important universal and reliable measure clinicians utilize to classify CP severity and its impact on physical function is known as the Gross Motor function Classification System (GMFCS) which was first discussed in 1997 by Palisano and researchers (Paulson et al., 2017) The various levels described in the GMFCS are shown in **Figure 4.** While this five-level scale was originally designed to access function in children aged 2 to 12 years old, the GMFCS was expanded by this team in 2007 to include adolescents between the ages of 12-18 (Paulson et al., 2017). Although the GMFCS is relatively stable across the life course for children, Palisano et al., found a repeated need for classification in children who were classified before the age of 4. (Palisano et al., 2018). If a child has a GMFCS level of I, II or III, the condition is also considered ambulatory CP and indicates ability to walk or having limited walking ability (Finbråten et al., 2015). A classification in children with a level of IV and V is known as non-ambulatory and includes limited mobile ability without a supportive device (Finbråten et al., 2015).

Another outcome measure to access impact of CP is the International Classification of Functioning, Disability and Health by the World Health Organization (ICF-WHO), intended for both children and adults (Schiariti et al., 2014). The ICF takes a dynamic approach when analyzing factors that influence daily functioning for children with CP (Schiariti et al., 2014). These factors include a variety of questions that analyze the individual's perception about their body function and structures, activities and

participation, personal values, and environmental factors that may intensify the secondary outcomes of disability (Schiariti et al., 2014).

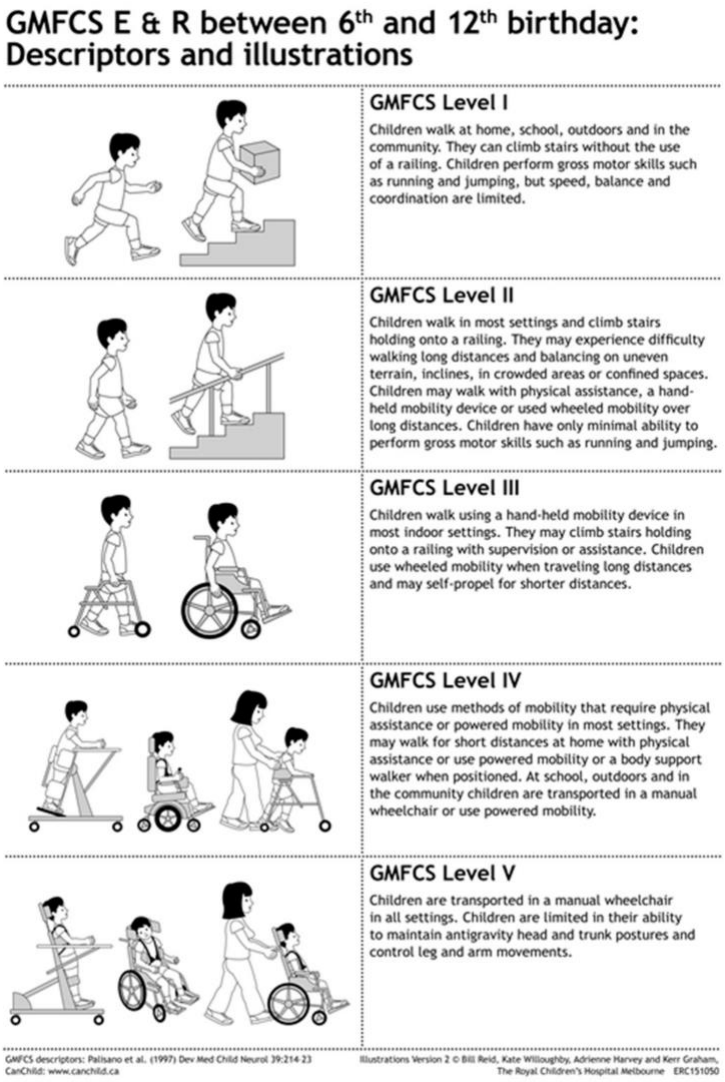


Figure 4: How the Gross Motor Function Classification System measures CP severity. The expanded and revised GMFCS scale that care providers use to access the impact of severity on function in individuals with CP. The GMFCS is a standardized and universal tool to improve communication and coordination between care providers. A child at level I and II can walk without an assistive device but children within levels 3-5 use a supportive device. Taken from (Paulson et al., 2017).

Common age for CP clinical diagnosis

According to Velde et al., various clinical presentations of CP, lack of biomarkers for CP and limited support for early inventions for CP creates barriers to confidently diagnosing a child with CP before 1 year of age (Velde et al., 2019). It is currently believed that early diagnosis is advantageous for maximizing neuroplasticity in children with CP, optimizing motor function and preventing severe secondary musculoskeletal impairment (Novak et al., 2017). Although CP is commonly diagnosed around 12-24 months of age in higher income countries, recent advances in clinical tools that are predictive of CP risk development has shifted diagnosis to as early as 5 months of corrected age. (Novak et al., 2017). The American Academy of Pediatrics calculates corrected age by "subtracting the number of weeks born before 40 weeks' gestation from the chronological age", which is also known as the age at birth (American Academy of Pediatrics, 2004).

Originally noted by Ashwal and researchers, the American Academy of Neurology endorsed the use magnetic resonance imagining (MRI) as a diagnostic tool for CP in 2004 (Velde et al., 2019). A systematic review by Novak et al., found that CP can be predicted with greater confidence before 5 months corrected age with MRI (86-89% sensitivity), the Prechtl Qualitative Assessment of General Movements designed in 2004 (GMA, 98% sensitivity) or the Hammersmith Infant Neurological Examination (HINE, 90% sensitivity) (Novak et al., 2017). The GMA is a questionnaire that identifies infants at risk for CP by watching their spontaneous movements (Aizawa et al., 2021). The

HINE is a 26-item examination that assess the cranial nerve function, posture, movements, tone, and reflexes in infants up to 24 months (Romeo et al., 2021). Novak et al., recommend the use of the HINE in lower- and middle-income countries if the use of MRI is not available (Novak et al., 2017).

CHAPTER TWO

Comorbidities secondary to CP

CP is associated with variety of conditions that places this population at higher risk of needing medical care when compared to the general population (Hollung et al., 2020). Although the primary condition of CP is the associated brain lesion which cannot be directly cured, the secondary, downstream conditions associated with CP can be managed through pharmacological and nonpharmacological methods. According to Patel et al., a multidisciplinary team of care providers, including an audiologist, medical social worker, nursing, nutritionist, occupational therapist, pediatrician, pediatric gastroenterologist, orthopedic surgeon, physiatrist, physiotherapist, psychologist, and speech-language therapist are needed to target the various associated comorbidities of CP and improve motor, tone and posture deficits as well as pulmonary health, communication skills, self-care, oral health, cognition and sleep habits that impact quality of life (Patel et al., 2020).

CP is characterized by a multitude of conditions depending on disease severity. The most common neurological condition associated with CP is epilepsy (Hollung et al., 2020). Visual and hearing abnormalities, sleep disturbances, pain, gastrointestinal and nutrition disturbances, respiratory conditions like pneumonia, and bladder incontinence are also common in this population (Pruitt et al., 2009). Some common secondary musculoskeletal conditions include upper and lower limb joint contractures, degenerative arthritis, scoliosis, and hip dislocation/displacement (Tonmukayakul et al., 2018).

Prevention Strategies to reduce pre-term birth and CP development

Promising prevention strategies focus on managing pathologies associated with preterm birth that can lead to CP development (Davidson et al., 2015). In a population-based Swedish study, birth weight of less than <10% for gestational age and prematurity were associated with increased risk for CP (Chen et al., 2022). The association has increased focus on treatment that protect fetuses from neurological insults that premature infants are increasingly susceptible to.

One line of prevention, administration of magnesium sulfate, was endorsed in 2010 by the American college of Obstetricians and Gynecologists as a fetal neuroprotector among mothers expecting an imminent pre-term birth (Committee opinion, 2010). One randomized controlled trial that compared mothers at risk of imminent birth before 30 weeks' gestation who received 4g of magnesium sulfate compared to mothers who received placebo found significant reductions in gross motor dysfunction and death among preterm infants (Crowther et al., 2003). However, overall reduction in CP development between these two groups did not approach statistical significance (Crowther et al., 2003).

In 2020, a systematic review with metanalysis and trial sequential analysis across 6 trials assessing the association between administration of magnesium sulfate to mothers during imminent preterm birth and reduction in CP development compared to mothers who did not receive magnesium sulfate had significant decreases in risk of CP with use of magnesium sulfate (Wolf et al., 2020). The use of magnesium sulfate in women with an imminent preterm birth of less than 32 weeks' gestation was found to be cost effective

from both a societal (quality of life years gained) and health care system cost savings perspective compared to lifetime cost of CP development if not prevented (Bickford et al., 2013). Additionally, the researchers found that administration of magnesium sulfate to women at risk of threatened birth, where preterm birth of less than 32 weeks was not certain to occur within 24 hours, was also cost effective from a societal perspective and somewhat from a health care system perspective (Bickford et al., 2013).

A preclinical study testing the use of therapeutic hypothermia, introduced in the 1990's, among hypoxia-induced piglets showed a reduction in pathological mediators of cerebral ischemia that included an overwhelming, toxic production of nitric oxide (measured as citrulline: arginine ratio) and extracellular excitatory amino acids (Thoresen et al., 1997, Shintaku et al., 2021). A review of 11 clinicals trials by Davison et al., has also supported the use of therapeutic hypothermia initiated within 6 hours of birth to reduce and potentially prevent severe CP in full term infants with hypoxic-ischemic encephalopathy, a risk factor for CP development (Davidson et al., 2015). The neurological impact of hypoxia-ischemia on brain development is shown in **Figure 5.** In 2010, the National Institute for Health and Clinical Excellence (NICE) based in England endorsed the use of therapeutic hypothermia in England, Wales, Scotland, and Northern Island in infants with hypoxic-ischemia encephalopathy (NICE, 2010).

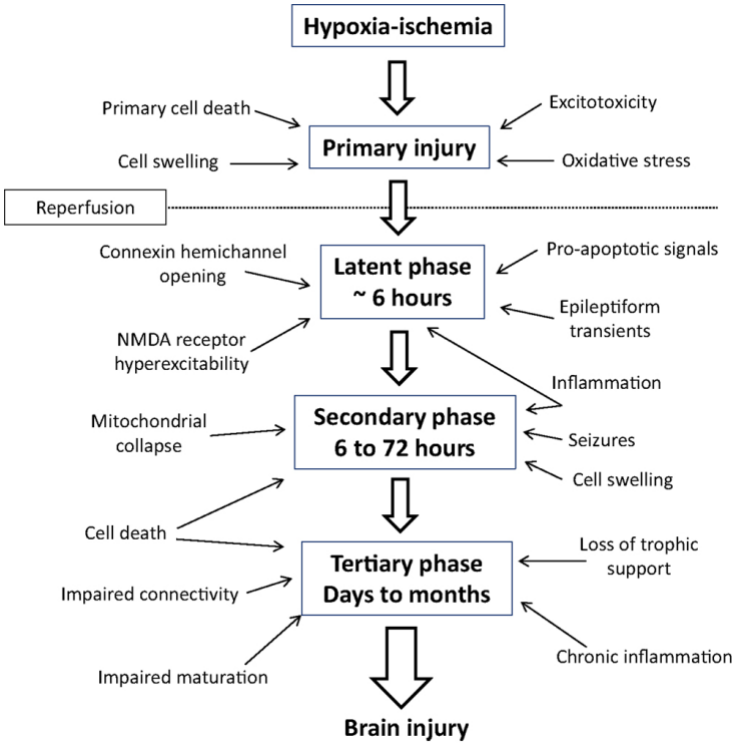


Figure 5: Cerebral injury associated with hypoxic-ischemia conditions. Cerebral cooling to 34 to 35 °C initiated within 6 hours is a protective method for full term infants with hypoxic-ischemia encephalopathy to manage inflammatory responses, cell death and prevent further neurological damage (Taken from Davison et al., 2015).

Non-pharmacological management of Cerebral Palsy

Orthopedic surgeons have an important role in the care and management of secondary conditions influenced by CP (Sharan et al., 2017). Some orthopedic interventions include osteotomies, arthrodesis (fusion of two or more bones in a joint), tendon transfers, and musculotendinous release and lengthening to minimize pain and reduce spasticity (Sharan et al., 2017). Individuals with CP often have muscle and bone length discrepancies that limit optimal functioning (Sharan et al., 2017). The single event multilevel surgery (SEMLS) has become a standardized method in the last two decades and targets multiple secondary musculoskeletal deformities such as muscle contractures

or muscle imbalance in children and adults with CP to prevent repeated hospitalizations for care (Sharan et al., 2017, Jea et al., 2019). Muscle contractures are defined by a shortening of a musculotendinous unit which prevents full range of motion (Lieber et al., 2019). However, lack of randomized control trials limits the comparison between SEMLS and other available treatments for CP (Jea et al., 2019).

Neurosurgical techniques have also become a popular method to manage spasticity in CP and increase range of motion specifically in the lower limbs of children (Peacock et al., 1991). Specifically, the selective dorsal rhizotomy (SDR) is a major technique that was revised and popularized into modern practice by Dr. Warwick Peacock from South Africa in the 1980's (Enslin et al., 2019). It is usually performed on children younger than 8 years old to optimize mobility (Aiudi et al., 2017). During the SDR procedure, dorsal sensory nerves are separated from the ventral motor nerves within the lumbosacral region (Jea et al., 2019). The sensory nerves are intraoperatively divided into sensory rootlets to test for irregular neurophysiological activity and those that demonstrate abnormal electrical activity in the muscle are cut as shown in **Figure 6** (Jea et al., 2019). An observational cohort study in England found that SDR improved function and quality of life in children with a GMFCS level of II and III, ultimately leading to the National Health Service's decision in 2018 to fund SDR's for this population (Summers et al., 2019). A retrospective analysis of 95 patients that underwent SDR 20-28 years prior to the study found no long-term complications related to the surgery (Park et al., 2017). Of these participants, 91% of participants believed the surgery

improved their quality of life compared to 2% who reported the opposite effect (Park et al., 2017).

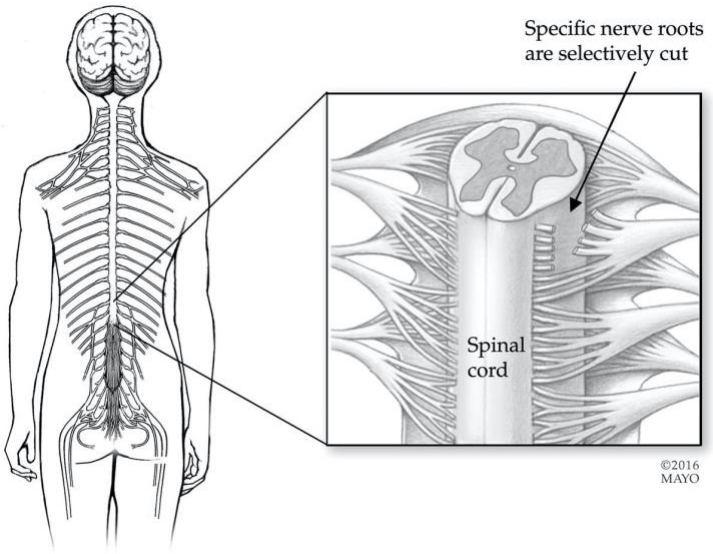


Figure 6: Sensory nerve cut during Selective Dorsal Rhizotomy procedure. Sensory rootlets that contribute towards spasticity are cut intraoperatively during the SDR. This neurosurgical procedure is commonly done on children younger than 8 years old. Taken from (Aiudi et al., 2017).

Most inventions to manage CP depend on severity and topographical regions that are impacted (Novak et al., 2020). While there are a variety of non-pharmacological treatments for CP in children, Dr.Iona Novak et al., created the Evidence Alert Traffic Light System in 2012 for rapid clinical decisions (Novak et al., 2020). The system functions to rate the quality of an intervention's outcome based on a traffic light's coloring system combined with the standardized Grading of Recommendations Assessment, Development and Evaluation (GRADE) System (Novak et al., 2020). The

GRADE system is a standardized framework that helps clinicians make decisions about efficacy (quality of evidence), applicability, benefits, and risks of an intervention (Atkins et al., 2004). According to Novak et al., an intervention that is assigned a green color indicates a high quality, effective study that often accompanies but not limited to randomized clinical trials (Novak et al., 2020). A yellow color indicates additional research is warranted to suggest effectiveness or conflicting findings exist in the literature (Novak et al., 2020). Lastly, a red light indicates the intervention can cause harm or is an ineffective treatment (Novak et al., 2020). Her research team has used the traffic light system to rate intervention commonly used for CP in children (Novak et al., 2020).

According to Novak et al., motor treatments for CP in children that are given a green light include action observation (improves hand function) , bimanual training (improves hand function), occupational therapy with botulinum toxin type A (works on goal achievement), environmental enrichment (improves hand function and gross motor function), Constraint-Induced Movement Therapy (improves hand function and participation), Hippotherapy, home program (improves hand function), partial body weight support Treadmill Training (improves walking speed), goal directed training (improves hand and gross motor function) strength training (improves muscle strength), mobility training (improves walking speed), task specific training (improves gross motor function), hip surveillance (reduces hip displacement), lower limb casting with prior Botox injections (providing passive range of motion) and treadmill training (improves walking speed, endurance and gross motor function) (Novak et al., 2020). Physical therapy aimed at improving overall strength through stretching as well as physical and

muscular range of motion plays a substantial role in treatment for children with CP (Franki et al., 2012). One form of physical therapy for CP includes treadmill training which is also discussed by Novak et al., (Franki et al., 2012, Novak et al., 2020).

Other inventions for cerebral palsy include use of external devices, such as ankle foot orthoses which help improve function or reduce muscle contractures that restrict range of motion (Wingstrand et al., 2014). The use of adaptive equipment, such as a wheelchair, can help increase participation in daily activities (Patel et al., 2020).

Pharmacological management of Cerebral Palsy

Pharmacological treatments for CP that come in both oral and interventional procedures and used to primarily target spasticity in children with CP (Chang et al., 2013). Continuing with the green light system used and created by Dr. Iona Novak et al., the use of botulinum toxin type A, intrathecal baclofen, and diazepam (an anticonvulsant) were found to be clinically successful in reducing spasticity (Novak et al., 2020). Botulinum toxin type A, one form of Botox, is used to address focal spasticity and its impact lasts for about 3-4 months (Chang et al., 2013). According to Kudva et al., Botulinum toxin type A, is often the first line of defense to manage spasticity associated with CP (Kudva et al., 2021). It is injected directly into affected muscles**,** inhibiting the secretion of acetylcholine from the pre-synapse, and preventing release at the neuromuscular junctions (Chang et al., 2013). Although botulinum toxin type A temporarily decreases spasticity, a systematic review across 15 randomized control trials

found there is limited evidence that supports its role in improving quality of life in children (Farag et al., 2020).

Another method to reduce spasticity is through Diazepam, a benzodiazepine that releases the inhibitory neurotransmitter gamma-Aminobutyric acid (GABA) on the post synaptic GABA-A receptor located in the central nervous system (Chang et al., 2013). However, a prospective randomized study found that while diazepam was associated with decreased spasticity and improved range of motion in children, significant adverse side effects included drowsiness and muscle weakness were also seen (Goyal et al., 2016).

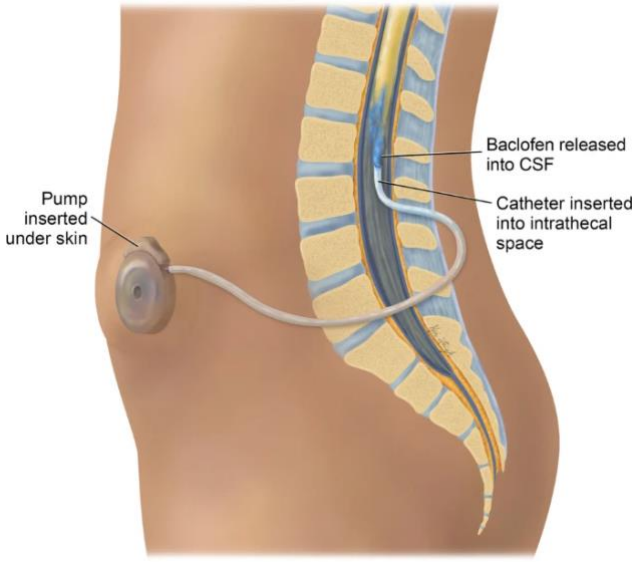


Figure 7: The location of the intrathecal baclofen pump. The intrathecal baclofen is used to manage severe spasticity through release of baclofen directly into the spinal cord. The pump is inserted into the abdominal region and is connected to the spinal cord via a catheter. Taken from (Kudva et al., 2021).

According to Dan et al., the intrathecal baclofen helps manage severe spasticity and is commonly used for children with a GMFCS level of IV and V (Stewart et al., 2017, Dan et al., 2010). The pump shown in **Figure 7**, which stores baclofen, is inserted into the abdominal wall (Stewart et al., 2017). The pump is attached to a Catheter which enters the intrathecal space where the cerebral spinal fluid is stored (Stewart et al., 2017, Kudva et al., 2021). GABA neurotransmitters are released onto GABA-B receptors within the spinal canal which results in hyperpolarization of the pre and post GABA-B receptors, inhibition of spinal reflexes and subsequently decreased spasticity (Stewart et al., 2017, Gracies et al., 1997).

Current research in treatment for CP

While pharmacological treatments for CP focus are focused on short-term management of the secondary manifestations associated with CP, recent clinical research has investigated various stem cell treatments as a cutting edge, "curative" therapy that focuses on long term neural repair, regeneration, and gross motor functioning in individuals with CP (Sun et al, 2010, Zakrzewski et al., 2019). In the next chapter, stem cells, preclinical models that support clinical translation of human stem cells in children, commonly used human stem cells for CP, domestic and international published clinical trials, systematic reviews and metanalysis of stem cell treatment will be reviewed that assess its efficacy and risks in clinical trials for children.

CHAPTER THREE

Introduction to stem cells

Stem cells are undifferentiated cells that make up the entire human body (Zakrzewski et al., 2019). The various types of human stem cells are shown in **Figure 8**. The totipotent cells have the greatest differential capacity and form the human zygote and extraembryonic structures like the placenta (Zakrzewski et al., 2019).

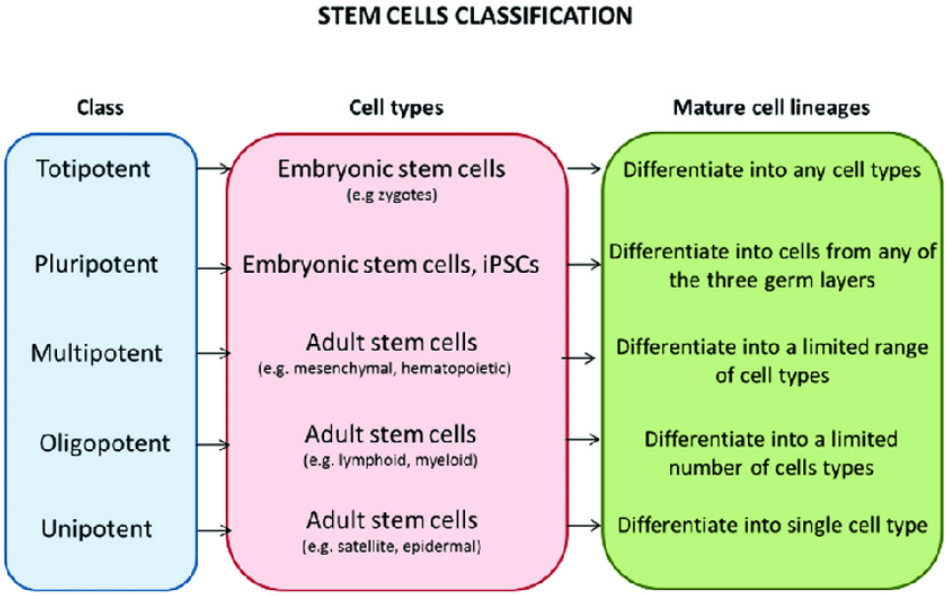


Figure 8: Examples of stem cell types. The classes of stem cells make up the human body and have unique functional and differential properties. Totipotent and unipotent cells have the greatest amount of cell differentiation ability. Taken from (Falzarano et al., 2019).

Totipotent cells form into pluripotent cells or embryonic stem cells which can differentiate into any cell lineage (Zakrzewski et al., 2019). In 2006, researchers Shinya Yamanaka and Kazutoshi induced pluripotent stem cells from fibroblast cells and found these new cells functioned similarly to pluripotent stem cells when induced by OCT 4,

sox 2, klf4 transcription factors or the myc gene (Zakrzewski et al., 2019). According to Ruff et al., totipotent cells are unsuitable for human transplantation because their high differential capacity increases the risk for teratoma and tumor development (Ruff et al., 2013). The National Cancer Institute defines a teratoma as a "type of germ cell tumor that may contain several different types of tissue, such as hair, muscle or bone" (National Cancer Institute, n.d). For similar concerns, pluripotent cells are not commonly used as a stem cell therapy (Ruff et al., 2013). Multipotent stem cells that are derived from pluripotent cells include cells that are restricted to certain lineages, such as the hematopoietic or mesenchymal stem cells and are the least likely to develop into teratomas (Zakrzewski et al., 2019, Ruff et al., 2013). Multipotent stem cell therapies are the most common form of stem cell therapy (Zakrzewski et al., 2019). According to researchers Rocha et al., stem cells are commonly derived from the bone marrow, peripheral blood, or the umbilical cord (Zakrzewski et al., 2019). Stem cells can also be derived from the placenta and adipose tissue (Ding et al., 2017, Sugiyama et al., 2018). Oligopotent cells can form various cells within a single lineage, such as myeloid stem cells. Lastly, unipotent cells only differentiate into a single cell type (Zakrzewski et al., 2019).

Human stem cell uses in hypoxia induced pre-clinical models

Preclinical animal models help elucidate etiologies behind a particular condition and test novel therapies for both efficacy and safety prior to clinical translation for humans. Although the preclinical models are typically animal models, they may provide

insight into finding the optimal timing of stem cell administration among known human hypoxic neurological insults (Ding et al., 2017, Sugiyama et al., 2018, Penny et al., 2020). While preclinical trials provide promising results in decreasing inflammation and increasing brain volume in induced brain hypoxia in an animal model, randomized clinical trials in humans are ultimately the gold standard to determine whether this treatment is effective.

Mesenchymal stem cells that are derived from the placenta, umbilical cord or Wharton's jelly are commonly used in preclinical models due to their evasion of the immune system, high proliferation, and high differential ability (Ding et al., 2017, Ruff et al., 2013). One clinical trial found a substantial increase in the number of inflammatory cytokines including TNF-α, INF-ϒ and IL-17 in hypoxic-induced rats pups compared to the rat pups' controls with no induced hypoxia (Ding et al., 2017). These researchers tested the protective effect of rat placental-derived mesenchymal stem cells (PD-MSC) with a dosage of 5 x 10^4 cells/10 μl transplanted 48 hours after inducing hypoxic-ischemia in rat brains (Ding et al., 2017). They found the rat pups with induced hypoxia and transplanted PD-MSC's had the greatest increase in Foxp3 gene expression, explained by researchers Ryba and Myśliwska in Poland as a transcription factor that regulates CD4+ and CD25+ T regulatory cells involved with reducing inflammatory markers such as TNF-α, INF-Y (Ding et al., 2017). These findings are shown in **Figure 9**. IL-10, an immunosuppressive cytokine, was also increased in the PD-MSC transplanted rat pups compared to the control rat pups, induced hypoxic-ischemia pups, and fibroblast treatment rat pups (Ding et al., 2017).

Umbilical cord blood cells (UCB) cells delivered via intraperitoneal and intranasal injections in hypoxic-induced neonatal rats with 1 or 3 doses of UCB cells infusion, compared to sham, control rats and hypoxic induced rats with no UCB cell infusion, found a significant increase in brain weight among rats with 3 doses of UCB cells compared to rats with only 1 dose of UCB cells infusion 40 days post hypoxia-ischemia induced injury (Penny et al., 2020).

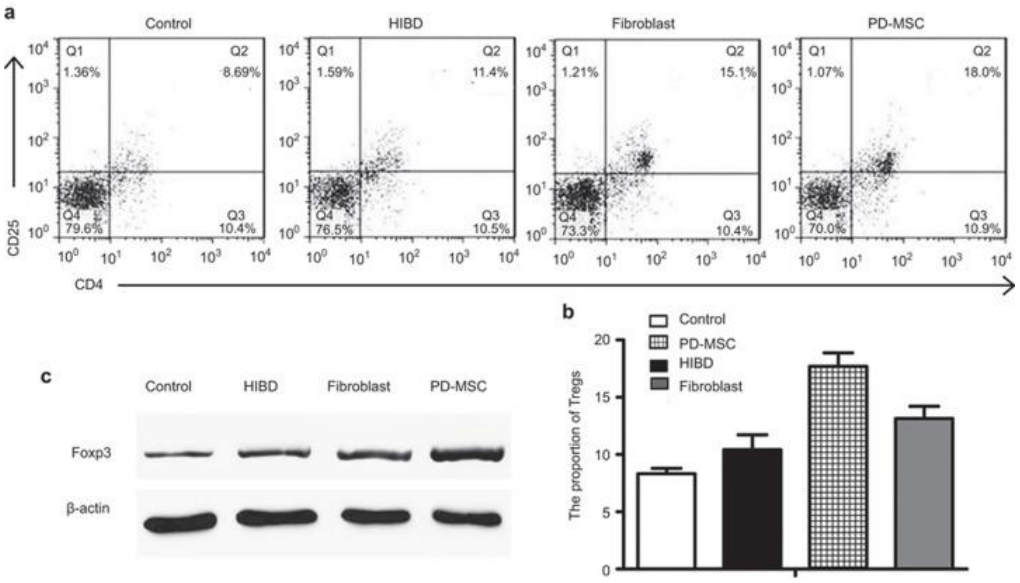


Figure 9: Data from Ding and researchers on placenta-derived mesenchymal stem cells transplanted into hypoxic induced rat pups. Figures c and b show Foxp3 gene expression, a transcription factor that regulates CD4+ and CD25+ T regulatory cells involved in the anti-inflammatory process was increased in hypoxic induced rat pups that had cerebral transplantation of placenta-derived mesenchymal stem cells (PD-MSC). Figure a is a flow cytometry graph of CD4+ and CD25+ cells and show the greatest number in the PD-MSC transplanted rat pups.

The three doses of intraperitoneal infusion consisted of 54 x 10^6/ kg, 40.5 x 10^6/kg and 27 x 10^6/ kg stem cells (Penny et al., 2020). Intranasal dosage consisted of 1 million

UCB during each administration (Penny et al., 2020) The single dose of UCB cells was initiated 24 hours after cerebral hypoxia was introduced while multiple doses of UCB cells were given at 24 hours, 72 hours, and 10 days after induction of cerebral hypoxia (Penny et al., 2020). Their results are shown in **Figure 10.** The variation in injection type did not result in a statistical difference in any outcome value tested in the study (Penny et al., 2020).

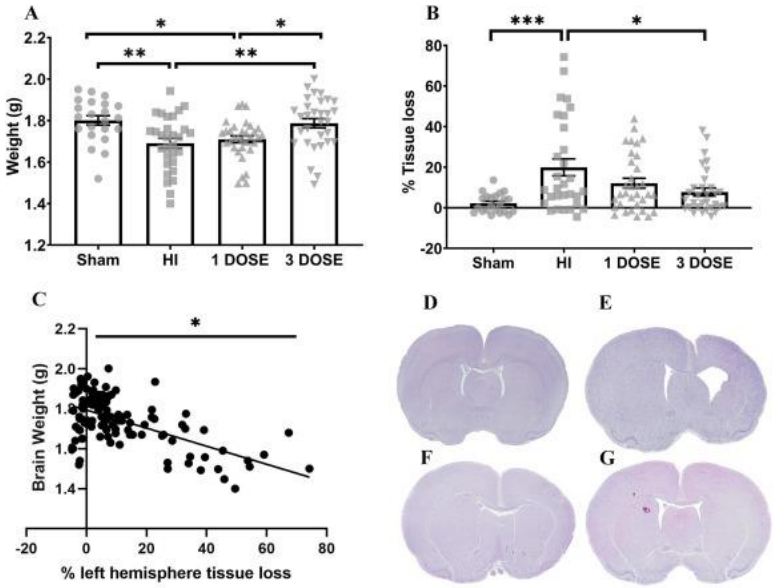


Figure 10: Data from Penny and researchers on umbilical cord blood cell transplanted into hypoxic induced rat pups. In 2020, Penny and researchers tested umbilical cord blood cells (UCB) in hypoxic-induced neonatal rats and included either 1 or 3 doses of UCBC infusion in rats, compared to sham, control rats and hypoxic induced rats with no UCBC infusion. Brain cut D = sham brain, E= hypoxic-induced neonatal rats, F= hypoxic-induced neonatal rats with 1 dose of UCB cells, G= hypoxic-induced neonatal rats with 3 dosages of UCB cells. Taken from (Penny et al., 2020).

43 days post hypoxic-ischemic insult the researchers found a significant decrease in the number of caspase-3 cell counts and activated microglia, suggestive of apoptosis and an inflammatory response following hypoxic-ischemia injury, respectively, among rats with

3 doses of UCB cell infusion compared to the hypoxic induced rats with no UCB cell infusion (Penny et al., 2020). This finding was associated with a decrease in neuronal cell death and inflammatory pathology in rats with 3 doses of UCB cell infusion (Penny et al., 2020).

The researchers also compared the hypoxic-induced neonatal rats with 1 or 3 doses of UCB cells infusion, compared to sham, control rats and hypoxic induced rats with no UCB cell infusion of rats through behavioral tests (Penny et al., 2020). Specifically, the behavioral outcomes among the novel object recognition test measuring short-term memory and exploratory behaviors found an increased score among rats with 3 doses of UCB cells when compared to hypoxic induced rats with no UCB cell transfusion (Penny et al., 2020).

Intravenous injection of bone marrow-derived MSC's (BM-MSC) and adipose tissue derived stem cells (ADSC) administered 4 or 24 hours after hypoxic cerebral injury in term rats found that those with ADSC infusion 4 hours after cerebral hypoxia had significantly higher mortality compared to rats with BM-MSC injection (Sugiyama et al., 2018). Rats were either injected with 1 x 10^5 cells or 1 x 10^4 stem cells on two separate occasions, or only a one-time injection of 1x 10^5 or 1 x 10^4 stem cells (Sugiyama et al., 2018). Rats injected with ADSC were found to have a greater severity of lung hemorrhaging and pulmonary embolisms, compared to rats with injected BM-MSC (Sugiyama et al., 2018). These results suggest variations in stem cell risk based on cell type. Rats with injected BM-MSC, but not ADSC, also had a significant increase in anti-inflammatory cytokine IL-2 levels and significant decrease in M1, microglial pro-

inflammatory pathway within the penumbra of the cerebral cortex, compared to control rats without induced hypoxic-ischemic (Sugiyama et al., 2018). The M1 microglial pathway involved in pro-inflammatory pathways is shown in **Figure 11** (Nakagawa et al., 2014).

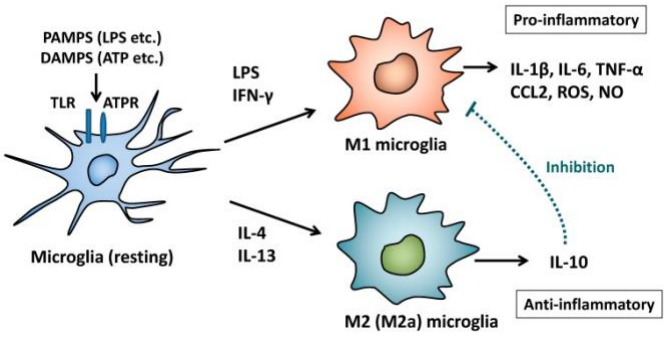


Figure 11: **The two pathways of microglial cells; M1 and M2.** The presence of certain mediators influences the activation of M1 microglial or M2 microglia. Taken from (Nakagawa et al., 2014).

Interestingly, both BM-MSC's and ADSC s were not located in the brain and were found in the lungs or liver of the rats (Sugiyama et al., 2018).

Although preclinical models help clarify the pathological insults within the brain and are used to better understand the mechanism of stem cells in hypoxic-ischemic, inflammatory conditions or other CP like conditions, there are limitations to these models (Brandenburg et al., 2019). Pre-clinical rat models of CP do not develop symptoms of spasticity, common seen in human CP, but continue to be a common model to study the effects of stem cell treatments (Brandenburg et al., 2019, Passera et al., 2021). In addition, due to a variety of CP etiologies, a variety of animal models that test stem cell therapies on specific cerebral insults are needed.

Human stem cells used in clinical trials for CP in children

Hemopoietic stem cells that are derived from the umbilical cord are currently the only FDA approved stem cell therapy since the 1950's and specifically target hematological diseases ([FDA], 2021, Sun et al., 2021). Additionally, there are currently no proven stem cell therapies for neurological disorders although they are currently under study ([FDA], 2021). A 2019 systematic review of neurological conditions that are registered in clinical trials include multiple sclerosis, stroke, spinal cord injury, cerebral palsy, amyotrophic lateral sclerosis, hypoxic ischemic encephalopathy, autism, Parkinson's disease, Alzheimer's disease, and ataxia (Alessandrini et al., 2019). Extensive safety and efficacy of stem cell treatment must be demonstrated prior to their clinical application in humans.

Cell Types

According to Sun et al., the focus of stem cell treatment in children is to initiate and maintain reparative mechanisms within the cerebral tissue via paracrine mechanisms, increase synaptogenesis and angiogenesis and minimize inflammation (Sun et al., 2021). Ideally, this combination would contribute to long term functioning and greater independence in children with CP. Pre-clinical animal trials have shown that stem cells promote various anti-inflammatory pathways in hypoxic-ischemic induced conditions that prevent further neuronal cell death and increase brain weight in these models (Ding et al., 2017, Penny et al., 2020). A variety of potential candidates for stem cell therapy in humans are shown **Figure 12.**

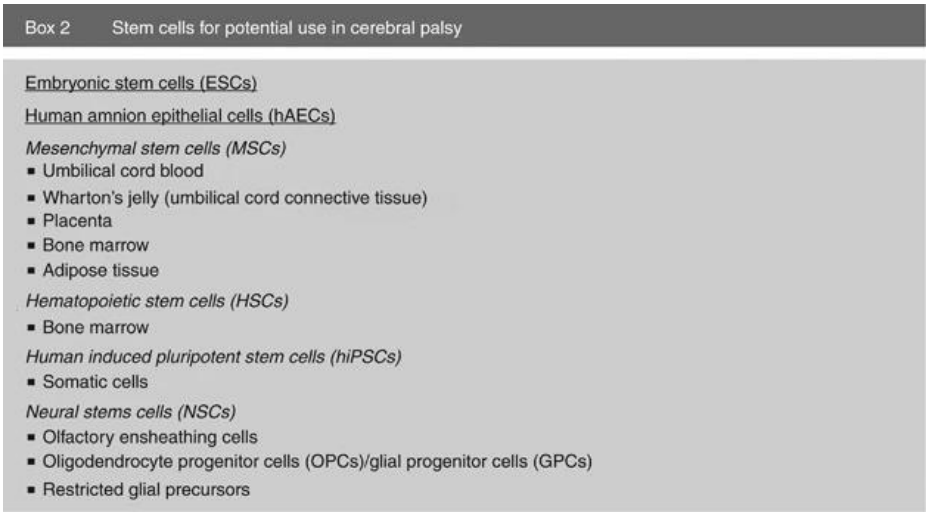


Figure 12: Various stem cells that have been studied for use in children with CP. Mesenchymal stem cells (MSC), specifically from umbilical cord, are commonly used in clinical stem cell trials due to their anti-inflammatory response, low immunogenicity, and ethical concerns. MSC's can also be derived from a variety of sources which makes them easily accessible and strong candidates for stem cell therapy. (Ruff et al., 2013, Penny et al., 2020) (Taken from Jantzie et al., 2018).

Mesenchymal stem cells and umbilical cord blood, which contain mesenchymal, hematopoietic, and endothelial stem cells, are commonly used in clinical stem cell trials (Passera et al., 2021). The use of embryonic and neural stem cells has raised important ethical concerns and are not commonly used in clinical trials or safety assessments (Passera et al., 2021). Human induced pluripotent stem cells are also not commonly used in clinical trials due to their embryonic stem cell like qualities and ability to form teratomas (Ruff et al., 2013). Additionally, while neural pre cursor cells would be ideal to promote neural growth and enhance neural connections, Ruff et al., note that their harvest is challenging due to their location in the adult brain (Ruff et al., 2013).

An estimate of the completed and active trials listed on clinicaltrials.gov over the past 15 years was compiled by Paton et al., (Paton et al., 2021). Their search did not find any published phase 3 trials and is shown in **Figure 13** (Paton et al., 2021). According to Passera et al., the most common routes of stem cell administration include intravenous and intrathecal infusion (Passera et al., 2021).

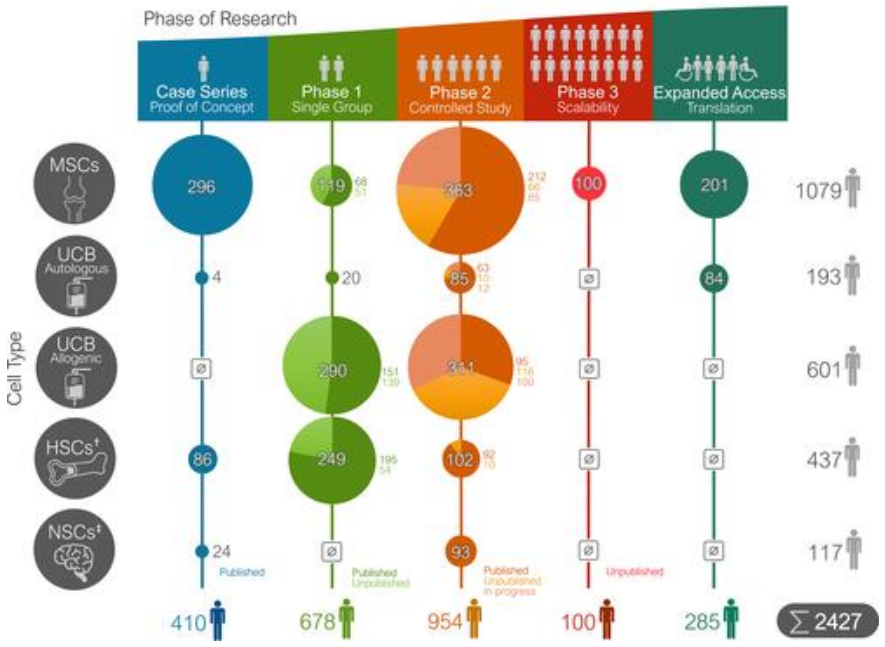


Figure 13: Overview of clinical trial on stem cell treatments for CP over the last 15 years. Cell types used in to assess safety, efficacy, and any improvements in secondary CP conditions. MSC= mesenchymal stem cells, UCB= umbilical cord blood, Autologous= cells obtained from self, Allogenic= cells obtained from another source, HSC= Hematopoietic stem cells, NSC= Neural stem cells. (Taken by Paton et al., 2021)

Properties of popular stem cells used in clinical trials for CP

Stem cells have specific reparative properties that make them compelling candidates for neurological conditions. Specifically, the umbilical cord blood (UCB)

carries a variety of cells involved in immunomodulation (McDonald et al., 2018). The various stem cells found in UCB are shown in **Figure 14.**

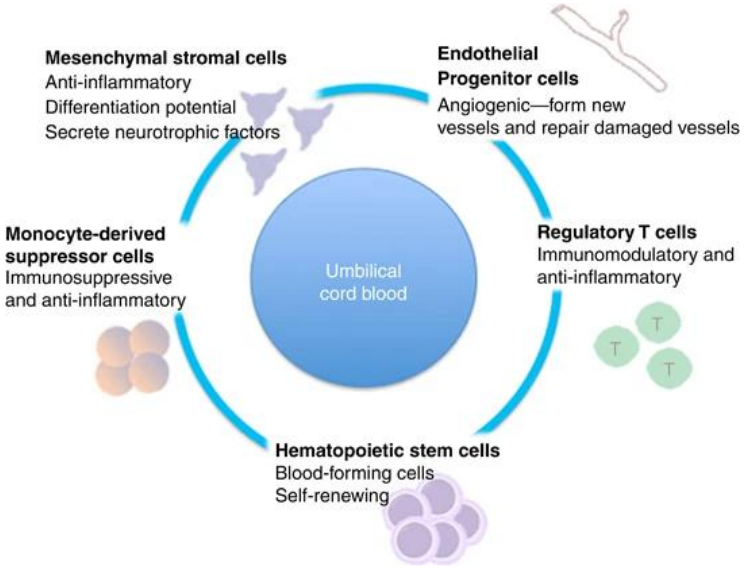


Figure 14: Stem cells within umbilical cord blood. The various stem cells within the umbilical cord with low immunogenicity properties make this an attractive candidate for potential stem cell treatment. (Taken from McDonald et al., 2018).

MSC's derived from the umbilical cord also have a higher proliferative and differential capacity compared to MSC's derived from the bone marrow, whose harvest is also associated with increased risk for infection (Ding et al., 2017). Additionally, MSC's from UCB are noted to have low immunogenic properties due to their lack or decreased number of major histocompatibility complexes (Sun et al., 2021). In addition to the umbilical cord, MSC's are found in Wharton's jelly, placenta, bone marrow, peripheral blood, and adipose tissue (Jantzie et al., 2013, Hass et al., 2011). The properties of mesenchymal stem cells are shown in **Figure 15.** The use of stem cell as a potential treatment for CP is suggested to improve the surrounding cerebral environment by

minimizing inflammation (Sun et al., 2021, Paton et al., 2022, Jantzie et al., 2018). The suggested mechanisms of action are shown in **Figure 16.**

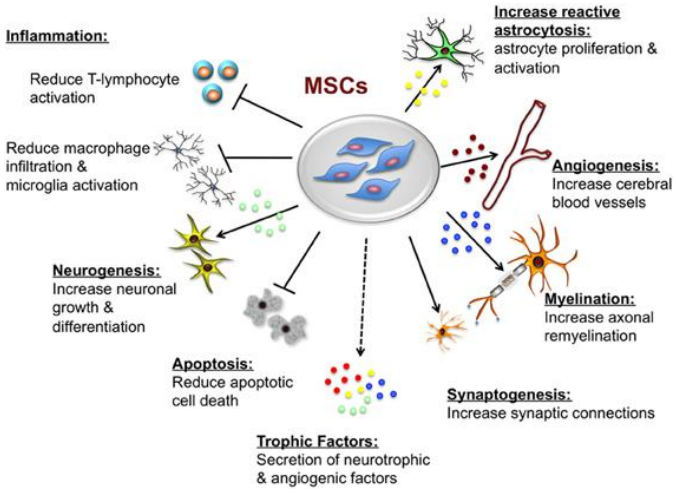
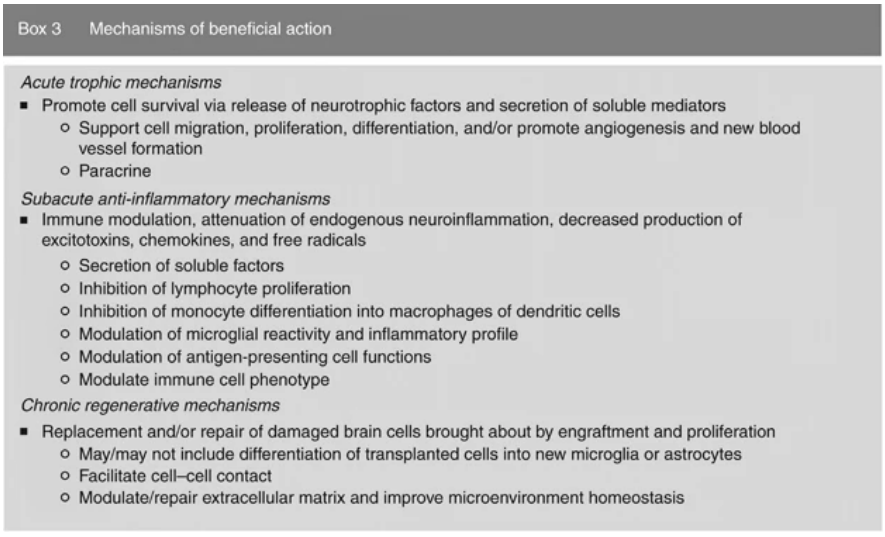


Figure 15: MSC stem cell properties. MSC's have a variety of beneficial impacts that make them popular candidates for CP. Taken from (Castillo et al., 2013).

Mechanisms of action



Box 3 Mechanisms of beneficial action

Acute trophic mechanisms

- Promote cell survival via release of neurotrophic factors and secretion of soluble mediators
  - Support cell migration, proliferation, differentiation, and/or promote angiogenesis and new blood vessel formation
  - Paracrine

Subacute anti-inflammatory mechanisms

- Immune modulation, attenuation of endogenous neuroinflammation, decreased production of excitotoxins, chemokines, and free radicals
  - Secretion of soluble factors
  - Inhibition of lymphocyte proliferation
  - Inhibition of monocyte differentiation into macrophages of dendritic cells
  - Modulation of microglial reactivity and inflammatory profile
  - Modulation of antigen-presenting cell functions
  - Modulate immune cell phenotype

Chronic regenerative mechanisms

- Replacement and/or repair of damaged brain cells brought about by engraftment and proliferation
  - May/may not include differentiation of transplanted cells into new microglia or astrocytes
  - Facilitate cell–cell contact
  - Modulate/repair extracellular matrix and improve microenvironment homeostasis

Figure 16: Suggested mechanisms that stem cells take in vivo. There are various pathways that stem cells may take to regenerate damaged neuronal tissue. (Taken from Jantzie et al., 2017).

Published literature on efficacy of stem cell treatments in children with CP

Safety profiles

Potential new treatments must go through extensive preclinical modeling to test for mechanisms of action and toxicology profiles prior to clinical translation in humans ([FDA], 2020). The process a new drug must take before clinical use is outlined by the US Food and Drug Administration (FDA) and show in **Figure 17.**

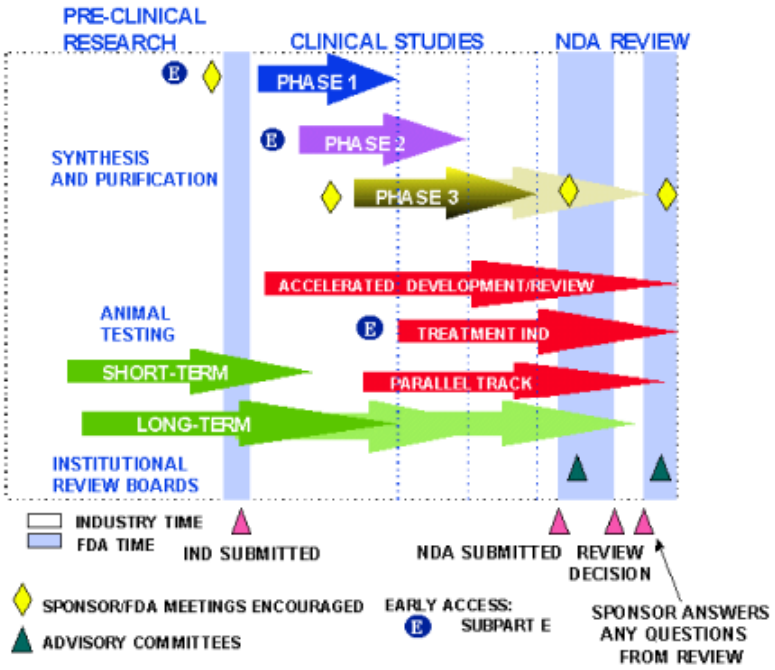


Figure 17: FDA approval process for new drug candidates. There are numerous clinical steps a new drug must take before it can translate into clinical care. Phase I and Phase II trials, which measure safety profile in humans and both safety and efficacy in a larger number of individuals, respectively, have been completed with stem cells in children with CP. IND= Investigational New Drug Application, NDA= New Drug. Taken from ([FDA], 2020).

Numerous clinical studies have demonstrated the safety of umbilical cord blood use in children with CP (Sun et al., 2020, Sun et al., 2021, Paton et al., 2021). A 2010 pilot

study tested intravenous autologous umbilical cord infusion in 184 children with acquired neurological diseases such as CP (Sun et al., 2010). In this study, autologous cells from UCB were mostly taken from a privately cord blood bank that stores cord blood units for autologous or family use and differs from a public cord blood bank which stores cord blood with specific banking criteria and is used for allogenic purposes (Sun et al., 2010, Armitage et al., 2016). According to Armitage et al., a cord blood bank is a highly regulated and accredited facility that stores UCB for future use in either a private or public bank (Armitage et al., 2016). However, the cord blood in public banks is held to a different cell criterion standard than those in a private bank (Armitage et al., 2016, Sun et al., 2010). The umbilical cord blood used in Sun and researcher's pilot study had to include a total nucleated cell count greater than 1 x 10^7 total nucleated cells / kg of UCB for intravenous infusion to occur (Sun et al., 2010). If umbilical cords contained an excess number of cells, a second infusion was given to select children between 6 and 12 months after the first infusion to assess a potential dose dependent response (Sun et al., 2010). No adverse events were reported in the 183 children studied over a median follow up period of 12 months (Sun et al., 2010).

The use of allogenic stem is also being studied for potential use in children with CP allowing a broader distribution secondary to increased availability of these cells. In a 2020 phase I trial, Sun et al., studied the infusion of umbilical cord blood which contained a cell dose greater than 2.5 x 10^7 total nucleated cells / kg of UCB from a related sibling in 15 children aged 1-6 that had spastic quadriplegia, tetraplegia, diplegia and hemiplegia (Sun et al., 2021). The cord blood from siblings was fully, or partially

human leukocyte antigen matched to the child undergoing intravenous infusion (Sun et al., 2021). The children undergoing treatment were not given any immunosuppressants prior to infusion (Sun et al., 2021). The researchers reported no adverse effects related to UCB infusion over a 2-year period, suggesting storage of umbilical cord blood should be considered for use as a standard of care (Sun et al., 2021).

In a 2020 randomized, controlled, double blinded clinical trial, 4.5-5.5 x 10^7 kg of human umbilical cord- MSC's (hUC-MSC's) were intravenously infused in children aged 2 and 12 years with CP (Gu et al., 2020). Over 12 month follow up period, the researchers found no significant difference in adverse effects between children who received hUC-MSC's, and rehabilitation compared to children who received rehabilitation and a placebo (Gu et al., 2020). A few cases of fever occurred in the interventional group but subsided within 2 days (Gu et al., 2020).

Larger trials begin once safety in humans is demonstrated during phase I trials. The next section will review phase I and phase II clinical trials for CP treatment in children and its impact on the Gross Motor Function Measure (GMFM), a main outcome measure that is used to address stem cell efficacy in children.

How efficacy of stem cell therapy is measured

Standardized outcomes within a clinical setting test for efficacy of a potential treatment. In the scientific literature, the GMFM is a standardized clinical measure for children aged 5 months to 16 years and assess changes in gross motor function in children with CP (Beckers et al., 2015). The GMFM consists of either the GMFM-66 or

GMFM-88, which assess development milestones across lying and rolling, sitting, crawling, and kneeling, standing, walking, running, and jumping in children on a 4-point Likert scale (Beckers et al., 2015). The numbers 66 or 88 refer to the number of items asked during the developmental assessment (Beckers et al., 2015). The GMFM-66 is meant to be administered without the use of assistive devices while the GMFM-88 can be administered with shoes and assistive care devices (Beckers et al., 2015).

Another common clinical measure is the Peabody Developmental Motor Scales-second edition (PDMS-2) originally developed by researchers Folio and Fewell (Zanella et al., 2021). According to Zanella et al., the PDMS-2 measures fine and gross motor function from birth to around 6 years of age (Zanella et al., 2021). These researchers note that each question is out of 0 to 2 points, where 0 points mean the developmental performance was not met during a specific task, 1 point to indicate partial developmental performance was met and 2 points indicates developmental performance was met optimally during task performance (Zanella et al., 2021). The Peabody Developmental Motor Scales- second edition (PDMS-2) also contains the fine motor quotient and gross motor quotient that complies scores from completed tasks across various developmental domains (Zanella et al., 2021). For example, Zanella et al., note that gross motor quotient score is a combined score across 30 stationary tasks, 89 locomotion tasks, 24 object manipulation tasks for children aged 12 months or over and 8 reflex tasks for children younger than 11 months (Zanella et al., 2021).

Efficacy of stem cells as treatment in children among phase I and II trials

Randomized clinical trials are a gold standard design that assess the impact of a potential treatment compared to another group that does not receive the same treatment or receives the current standard of care. In a 2017 double blind, randomized, placebo controlled cross over clinical trial in the United States, Sun et al., tested the association between autologous cord blood infusion in 63 children with CP aged 1-6 and impact on gross motor function and brain connectivity (Sun et al., 2017). The type of CP in these children ranged from spastic diplegia, hemiplegia, quadriplegia and 1 child with hypotonic quadriplegia (Sun et al., 2017). Children were either randomized to a placebo group or autologous cord blood group, that contained a low infused stem cell dose of <2 x 10^7 total nucleated cells / kg or high dose of stem cells ≥2 x 10^7 total nucleated cells / kg (Sun et al., 2017). From baseline to the 1 year follow up, there was no significant difference in GMFM-66 scores when comparing the placebo group to children who received a low infused stem cell dose of <2 x 10^7 total nucleated cells / kg (Sun et al., 2017). However, there was a statistically significant difference in GMFM-66 scores when comparing the placebo group to children who received a high infused stem cell dose of ≥2 x 10^7 total nucleated cells / kg during 1 year of follow up (Sun et al., 2017). Additionally, the PDMS-2 gross motor quotient score was significantly higher among the high infused stem cell dose group when compared to the placebo and low infused stem cell dose groups (Sun et al., 2017). The physicians or therapists who performed the GMFM and PDMS-2 were blinded to the treatment group of each participant (Sun et al., 2017). The results from their study are shown in **Figure 18.**

Sun and researchers also obtained data on GMFM-66, PDMS-2 and overall brain connectivity measured through magnetic resonance imaging (MRI) 1 year after the intervention (Sun et al., 2017). This data would help researchers better assess if the impact of treatment was still impactful long term.

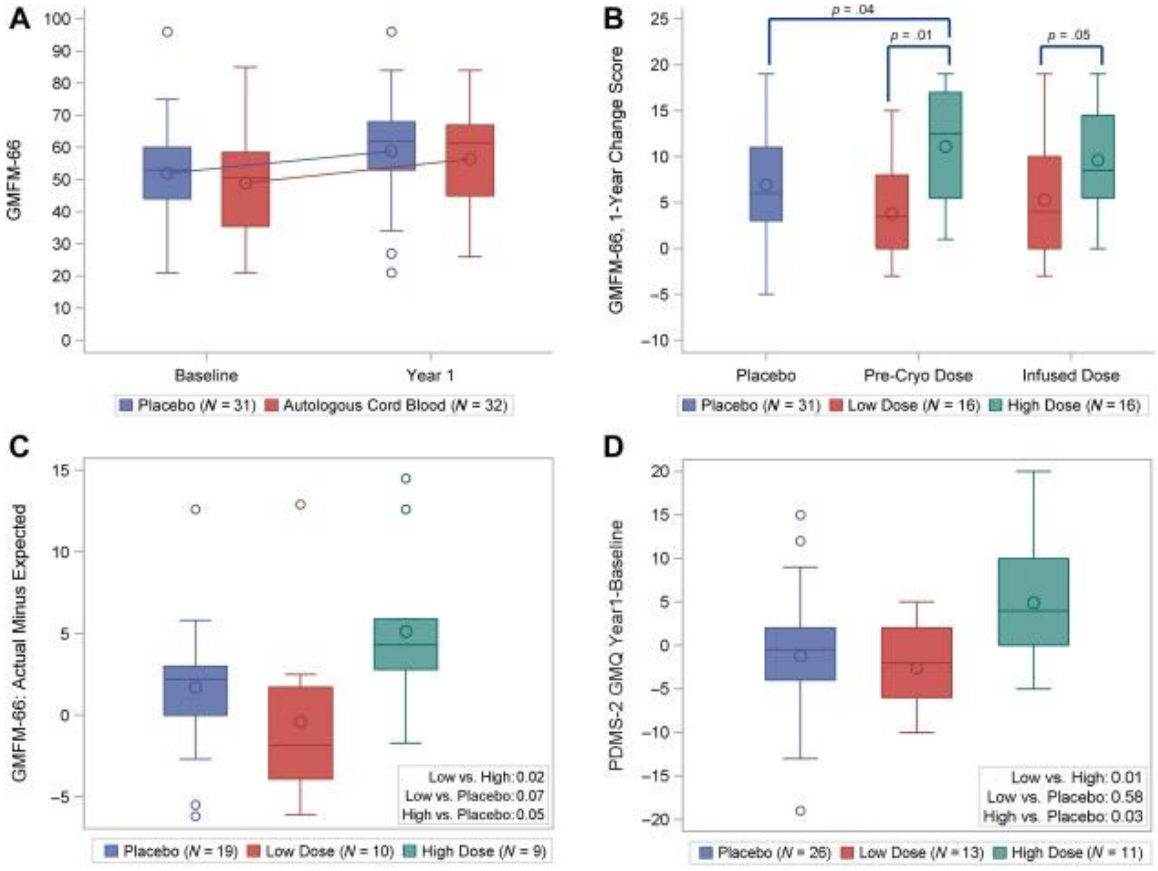


Figure 18: Randomized placebo controlled clinical trial data from Sun and researchers on human autologous cord blood infusion in children at 1 year follow up. Two doses of autologous cord blood were compared to a placebo group in children with CP to assess impact on gross motor function. A high infused stem cell dose of ≥2 x 10^7 /kg was associated with significant increases in Gross Motor Function Measure (GMFM-66) and PDMS-2 (Peabody Developmental Measurement Score-2 (PDMS-2) scores in the high dose stem cell group compared to the low dose stem cell <2 x 10^7 /kg and placebo group from baseline to 1 year of follow up. Taken from (Sun et al., 2017).

At that time, the researchers found a positive and statistically significant difference in GMFM-66, PDMS-2 measure after the 1 year follow up and brain connectivity, measured at the 2 years follow up when comparing participants in the high dose vs the low dose group (Sun et al., 2017). In this case, brain connectivity is comprised by nodes in the gray matter brain regions (Sun et al., 2017). According to these researchers, the "connectivity from any given node, or between any pair of nodes…was first measured by determining volumes of relevant white matter fiber pathways projecting from that node or between a pair of nodes" (Sun et al., 2017). These results are shown in **Figure 19.**

A 2018 randomized, placebo-controlled, single-blinded clinical trial in China assessed the combined impact of infused allogenic human umbilical cord mesenchymal stem cells (hUCB-MSC) and basic rehabilitation on gross motor and comprehensive functions in children with CP (Huang et al., 2018). 56 children with CP between the ages of 3 and 12 years old were eligible to participate in the study (Huang et al., 2018). The initial intravenous infusion of 5 x 10^7 hUCB-MSC /kg mixed in with 30mL 0.9% normal saline were given to children 1 day after randomization and the last three infusions were given on intervals of 7 days (Huang et al., 2018). The treatment group received infusion of hUCB-MSC in addition to basic rehabilitation while the control group received basic rehabilitation and normal saline (0.9% NS) (Huang et al., 2018). Additionally, rehabilitation sessions occurred twice each day for 1 hour only during the period that treatment or placebo was administered (Huang et al., 2018).

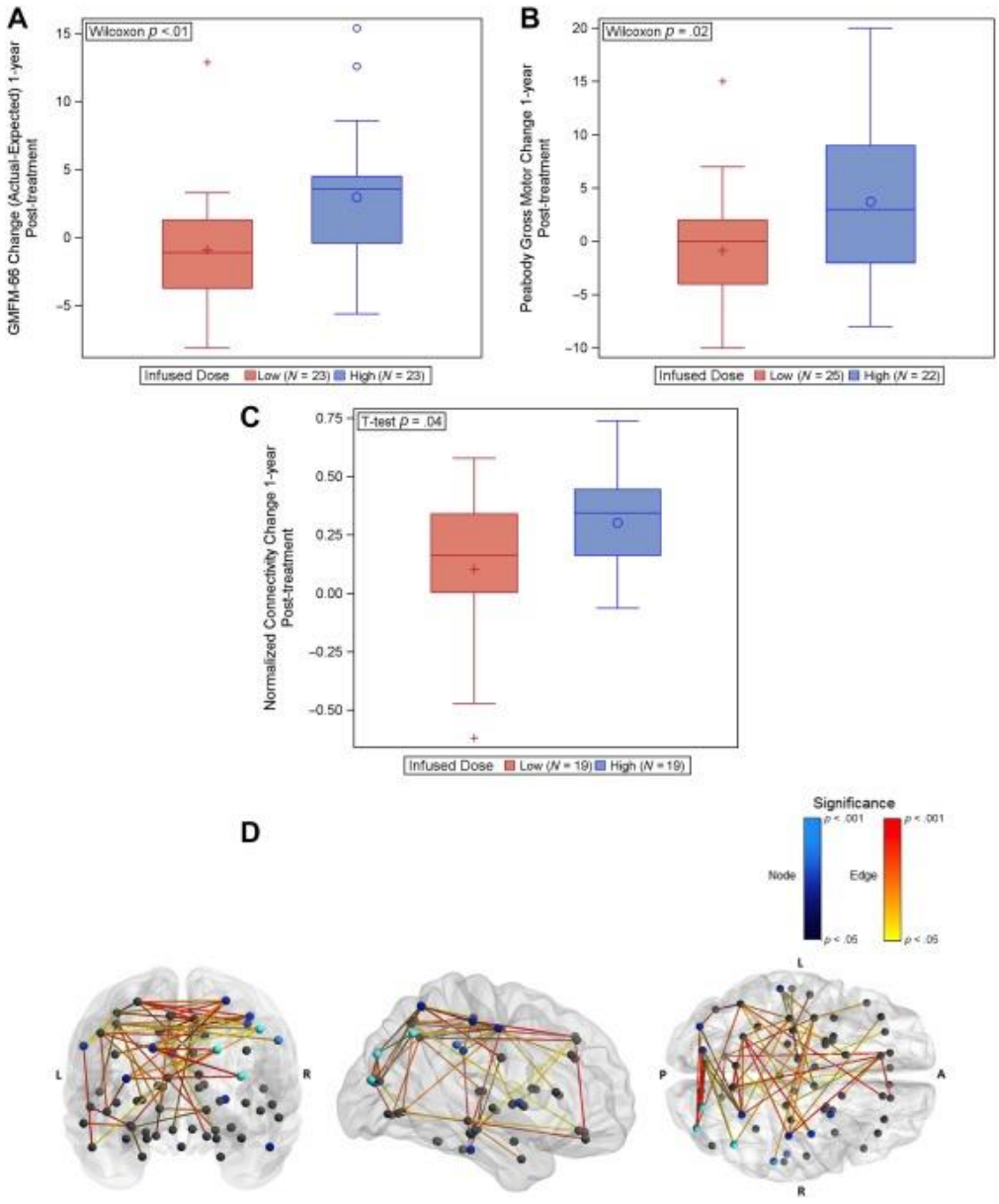


Figure 19: Randomized placebo controlled clinical trial data from Sun and researchers on human autologous cord blood infusion in children at 1 year post treatment. The clinical impact of a low or high dose autologous cord blood were compared in children with CP to assess impact on gross motor function. A high infused stem cell dose was associated with significant increases in GMFM-66 and PDMS-2 scores compared to children with the low dose stem dose 1-year post-treatment. At two years post treatment, image D depicts new brain connectivity, shown by the colored nodes which correlated to improvement in the GMFM-66 scores among children who received the high dose compared to a low stem cell dose. Gray nodes are non-significant and had no impact on increases in GMFM scores. Taken from (Sun et al., 2017)

The main outcome measures were measured at baseline, 3, 6, 9, and 12 months and included the GMFM-88 which is made up of 88 questions and the comprehensive functional assessment (CFA) (Huang et al., 2018). According to Huang et al., the CFA test measures cognizance, language competence, self-care, motor function and social adaptability (Huang et al., 2018). No serious adverse reactions or immunological rejections were reported during the study period (Huang et al., 2018). There was a statsitically significant increase and association on GMFM-88 scores among children that received hUCB-MSC's, and basic rehabilitation compared to children who only received basic rehabilitation (Huang et al., 2018). These results are shown in **Figure 20.**

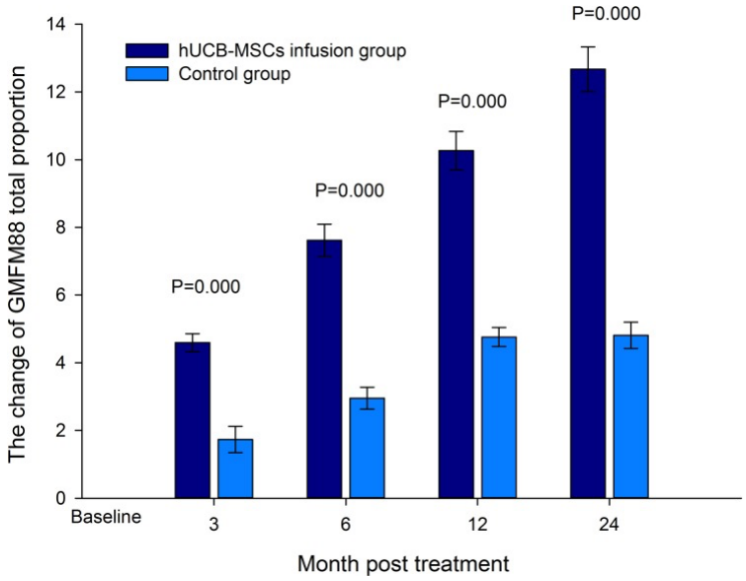


Figure 20: **Randomized placebo controlled clinical trial GMFM-88 score data from Huang and researchers among children with human umbilical cord blood mesenchymal stem cell infusion.** GMFM-88 score change at 3,6, 12- and 24-months post treatment among children aged 3-12 with CP that received the umbilical cord blood mesenchymal stem cell infusion was statistically significant compared to the placebo group. Taken from (Huang et al., 2018).

Additionally, their was a statsitically significant increase and association in CFA scores among children that received hUCB-MSC's, and basic rehabilitation compared to children who only received basic rehabilitation (Huang et al., 2018). These results are shown in **Figure 21.** The researchers concluded that these findings were clinically significant (Huang et al., 2018).

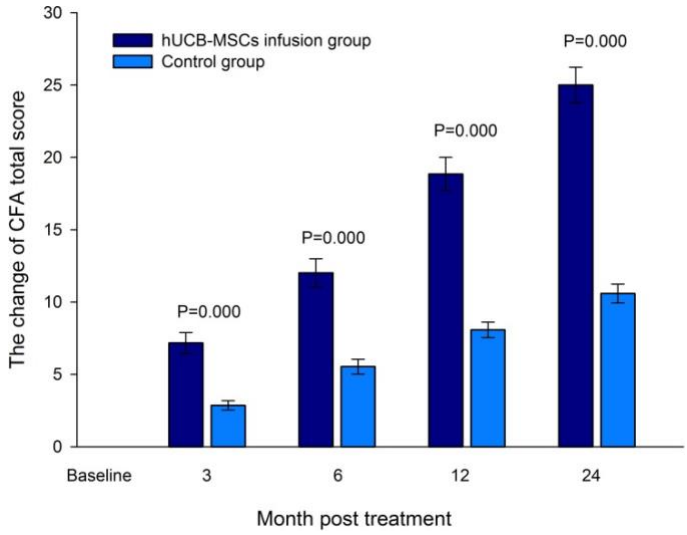


Figure 21: **Randomized placebo controlled clinical trial comprehensive function assessment score data from Huang and researchers among children with human umbilical cord blood mesenchymal stem cell infusion.** CFA score changes at 3,6, 12- and 24-months post treatment among children aged 3-12 with CP that received the umbilical cord blood mesenchymal stem cell infusion was statistically significant compared to the placebo group. Taken from (Huang et al., 2018).

Some researchers from this team performed an additional randomized, placebo-controlled, double blinded study in 2020 in China that further assessed the safety and impact of four intravenous infusions of 4.5-5.5 x 10^7 kg per unit of allogenic hUCB-MSC's with 50ml normal saline and 1% human serum albumin alongside rehabilitation verses solely rehabilitation on outcome measurements such as GMFM-88, CFA, and activities of daily living (ADL) scores in children with CP (Gu et al., 2020). According

to Gu et al., the ADL measures the ability to initiate personal care practices such as hygiene, feeding, dressing, toileting, ambulating, using tools, communication, position holding in bed and walking (Gu et al., 2020). Children with CP aged 2 to 12 years old were eligible for the study (Gu et al., 2020) and 39 ended up enrolling (Gu et al., 2020). Follow up outcome assessments were provided at 1,3,6 and 12 months after the last cell dose infusion (Gu et al., 2020). No significant adverse differences or any severe adverse reactions were found among children that received the stem cell intervention alongside rehabilitation compared to children who only received rehabilitation (Gu et al., 2020). Adverse events included low grade fevers, which typically subsided within 2 days, and were believed to be related to hUC-MSC infusion (Gu et al., 2020).

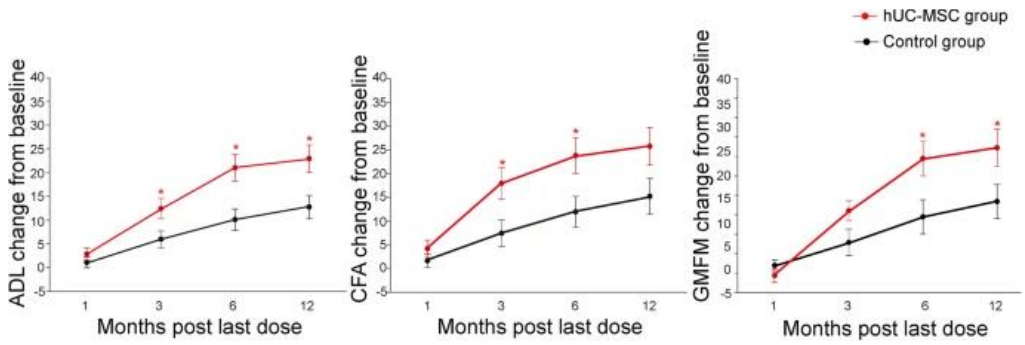


Figure 22: **Randomized placebo controlled clinical trial outcomes measures from Gu and researchers among children with human umbilical cord blood mesenchymal stem cell infusion.** Activities of daily life, comprehensive functional assessment gross motor function measure were the main outcome measures. The stars on the graphs represent a statistically significant value of $p<0.05$ when comparing children that underwent allogenic human umbilical cord mesenchymal stem cell infusion and rehabilitation compared to children who only underwent rehabilitation (placebo group). Taken from (Gu et al., 2020).

The results of the study are shown in **Figure 22.** At 3-, 6- and 12-months since the last dose significant improvements in ADL were observed while significant changes in

GMFM scores from baseline were seen at 6 and 12 months (Gu et al., 2020). However sustained improvement in CFA was not observed by 12 months after the last dose was administered (Gu et al., 2020).

Overall, significant improvements in GMFM scores and safety of stem cell interventions specifically from the umbilical cord were reported in children across the ages of 1 to 12 (Gu et al., 2020, Huang et al., 2018, Sun et al., 2017). While no long-term data or follow up studies are currently available among these children, the impact of stem cell interventions with autologous or allogenic cell dosage ranging from slightly less than 2 x 10^ 7 kg/ unit of UCB to 5.5 x 10^ 7 kg / unit of UCB lasted into 12 or 24 months follow up among GMFM measurements. Although this intervention is promising in children in terms of a benefit, risk assessment and larger clinical trials are needed to better understand if this intervention is the least invasive, most cost-effective and efficient path to permanent increases in gross motor function among children with CP.

Risks associated with stem cell treatment for CP in children

No severe adverse reactions in children were reported in any of the randomized clinical trial literature articles described previously (Gu et al., 2020, Huang et al., 2018, Sun et al., 2017). However according to various researchers, the suggested age and CP type, timing and method of administration, dosage, long term effects, and most efficacious type of stem cell for clinical use remains widely unknown (Jantzie et al., 2018, McDonald et al., 2018, Ruff et al., 2013). In this literature review of preclinical models, only ADSC were associated with adverse reactions, including a greater number

of lung hemorrhages and pulmonary embolisms, compared to rats with injected BM-MSC (Sugiyama et al., 2018). Hopefully with a greater number and size of randomized clinical trials researchers can move closer to answering these questions.

Alongside the rise of stem cell use came an increase in global stem cell tourism, which are made of clinics that promote unauthorized, unregulated and potentially unsafe means to receive stem cell treatments for a variety of conditions, ultimately taking advantage of vulnerable individuals looking for an immediate cure (Bauer et al., 2018). According to Bauer et al., these clinics are for-profit, utilize various stem cells for treatment and commonly infuse the stem cells intravenously or intrathecally, similar to the method used in regulated randomized clinical trials (Bauer et al., 2018). However, these treatments are associated with an increase in serious adverse reactions, such as loss of vision, neoplasm formation, infection, and death (Bauer et al., 2018). Lack of scientific endorsement, oversight or regulation ultimately shifts the balance to favor risks over potential benefits which may place patients under direct harm (Bauer et al., 2018). These clinics should require scientific endorsement and oversight by professionals to ensure patients are safe. Ruff et al., indicate that the cost of treatment in these clinics are around US $15,000-30,000 per treatment (Ruff et al., 2013).

Aleynik et al., note that stem cell delivery through the blood brain barrier (BBB), shown in **Figure 23**, is challenging due to its high selectivity and regulation (Aleynik et al., 2014). Although the researchers note that MSC's have crossed the BBB in other literature studies, their mechanism of action within various administration sites remains understudy (Aleynik et al., 2014).

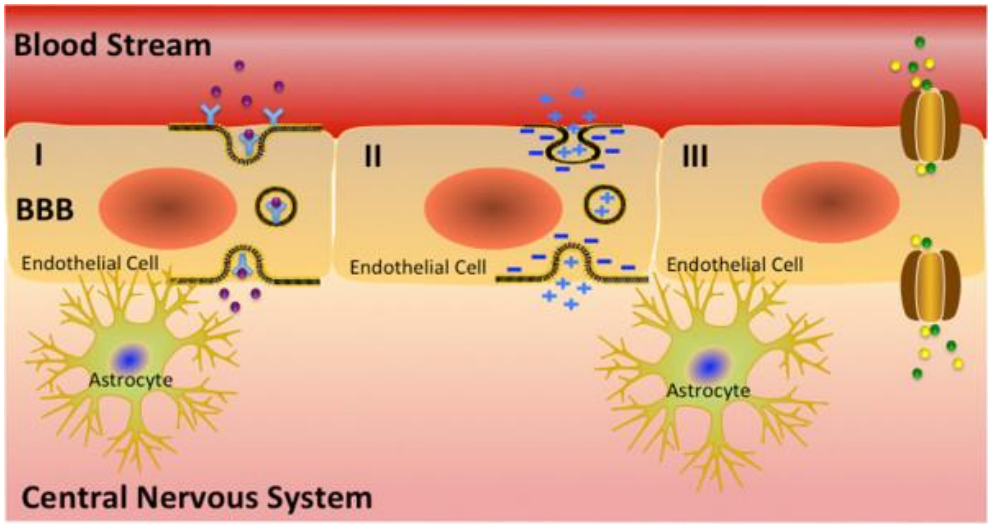


Figure 23: **Blood Brain Barrier (BBB)**. The BBB is a highly selective barrier, with a variety of protective mechanics that make stem cell migration to the injured brain regions more challenging. Taken from (Aleynik et al., 2014).

Cost

The cost of umbilical cord blood banking and stem cells as treatment also cannot be overlooked. Although Kaimal et al., argued that storage of umbilical cord blood is cost effective for high-risk children in need of a future stem cell transplant, storage for non-high-risk children is not as cost-effective (Kaimal et al., 2009). Additionally, the researchers note that initial umbilical cord blood storage fees can be as high as $4,170 USD (Kaimal et al., 2009). It is unclear whether this form of treatment would be reimbursed by insurance companies because stem cell therapy for children with CP is not approved by the FDA and is not a current standard of care. Lack of reimbursement may exacerbate out of pocket spending and ultimately exacerbate stress among certain families. Through Medicaid each state has the autonomy to decide if it covers stem cell

treatments (Preussler et al., 2014). Hopefully an additional number of efficacious phase II and III clinical trials can shorten the time to standardized coverage for stem cell treatment in children with CP.

CHAPTER FOUR

Future focus on prevention strategies

Prevention efforts, such as administration of intravenous magnesium sulphate to women at risk of preterm, imminent birth in addition to therapeutic hypothermia remain promising clinical efforts to reduce development of cerebral palsy (Wolf et al., 2020, Bickford et al., 2013, Thoresen et al., 1997, and NICE, 2010). From a public health perspective, these prevention tools target upstream factors that would ideally prevent adverse health outcomes downstream, associated with greater costs to the healthcare system (Pulgar et al., 2019, Bickford et al., 2013). The public health concept of targeting upstream factors is shown in **Figure 24.**

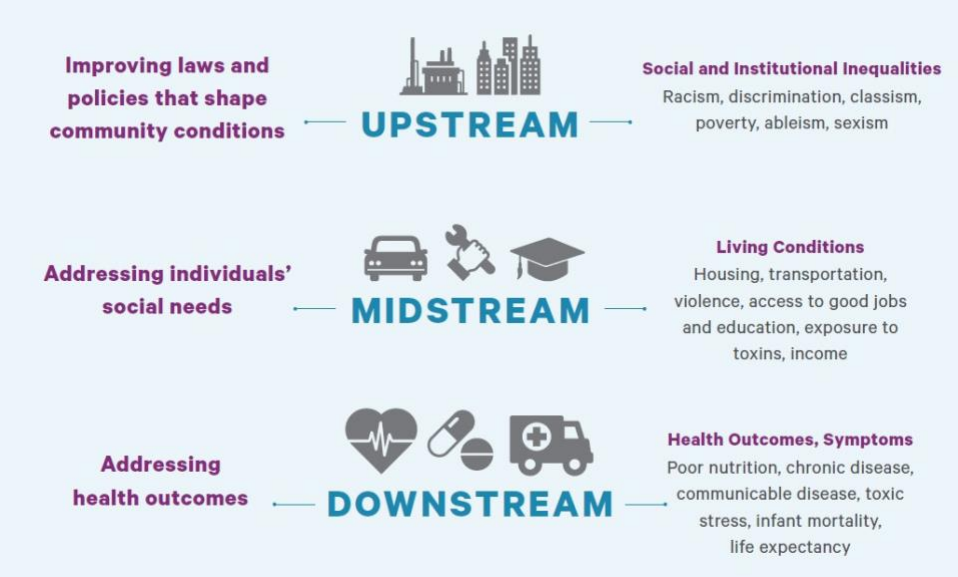


Figure 24: **How upstream factors impact health outcomes**. In public health, the terms upstream and downstream are commonly used to refer to ways structural and environmental inequities impact health outcomes, such as life expectancy, quality of life or development of disease. Ideally, public health interventions at the upstream level are critical for prevention of worse health outcomes and should be the focus for prevention of CP alongside greater exploration of stem cell treatment. (Taken from Ching, 2020)

Upstream factors refer to structural and environmental conditions, social inequities such as lower socioeconomic status or education and harmful policies that impact marginalized communities while downstream factors represent overall health outcomes (Ching, 2020). In addition to finding methods to potentially increase quality of life in children with CP, upstream factors that are associated with preterm birth or birth complications should be at the forefront of prevention. In a qualitative study in Serbia, researchers assessed the association of premature birth on development of cerebral palsy among 145 children (Demesi-Drljan et al., 2016). The researchers found that among these 145 children with a cerebral palsy diagnosis, 54.4% of them were born prematurely (Demesi-Drljan et al., 2016). While a variety of upstream factors exist, certain factors that include access to prevention services during prenatal/perinatal care and prior maternal health history or environmental stressors that may increase risk of premature births and subsequently increase risk for development of cerebral palsy should continue understudy.

Conclusion

Although individuals with cerebral palsy face physical limitations, their ability to positively influence the world has no limitations. They continue to make an extraordinary impact in our world, serving in professional careers and advocating for themselves and other individuals with disabilities, to name a few.

Stem cell interventions are most likely targeted in children with CP to theoretically optimize neuroplasticity and increase long-term repair (Novak et al., 2017). Although preclinical models usually test stem cell treatments after a few hours or days of induced hypoxia, the youngest aged human participants to receive this intervention were 1 year of age (Ding et al., 2017, Sugiyama et al., 2018, Penny et al., 2020, Sun et al., 2017). The most common stem cells found in the literature search that were used in children were derived from autologous and allogenic umbilical cords, known for protective properties and low immunogenicity (Gu et al., 2020, Huang et al., 2018, Sun et al., 2017, Ruff et al., 2013, Penny et al., 2020).

Current standard treatment for CP targets the musculoskeletal deficiencies associated with the condition. While diazepam, injection of botulinum toxin type A and the intrathecal baclofen pumps are noted to reduce spasticity in children, there have been conflicting evidence around their ultimate effectiveness and require continued administration and use (Kudva et al., 2021, Chang et al., 2013, Stewart et al., 2017). The exception to these short-term treatment options includes the use of the selective dorsal rhizotomy procedure which has been shown to improve quality of life up to 28 years after the initial surgery (Park et al., 2017). In addition to these treatments, continued expansion of prevention efforts, such as the use of magnesium sulfate, therapeutic hypothermia, and perinatal care guidelines to prevent premature births from occuring or from developing into cerebral palsy remains paramount.

Future, and larger human stem cell clinical trials are needed in children to answer lingering questions that concern the use of stem cells as a standardized of care and

whether they offer significant clinical and economic benefit to young children and their families compared to current treatments.

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