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Potential Of a Stem Cell-Based Therapy to Reverse Neurodegeneration from Spinal Muscular Atrophy

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ABSTRACT

Spinal Muscular Atrophy is a devastating neurological disease that primarily damages motor neurons, but has pathological impacts throughout the body. While current treatments of SMA can prevent further deterioration, a therapy to reverse motor neuron degeneration has not been developed. Stem cell-based transplantation offers the potential to replace lost neurons and reinnervate skeletal muscles, creating a degree of disease amelioration higher than ever before possible. Neural stem cells can provide neuroprotection to endogenous motor neurons through the secretion of neurotrophic factors while differentiating into a small number of motor neurons in vivo. They can be effectively cultured from pluripotent stem cell sources for transplantation. On the other hand, neural stem cells can also be differentiated into motor neurons in vitro and then directly injected into spinal cord parenchyma. However, various challenges must be overcome for this treatment to reach its full therapeutic potential. Overall, in animal models, cell migration to areas of neurodegeneration has been limited, and engraftment may be further hindered by signals from the spinal cord microenvironment, especially in a neurological disease such as SMA. Additionally, axon growth from transplanted cells is restricted and does not result in significant neuromuscular junction formation. There are potential solutions, however, such as alleviating inflammatory glial signals through SMN protein correction using current SMA treatments, thus increasing the engraftment rate, or enhancing axon elongation through hindering effects of myelin proteins using rolipram. Further research is needed to overcome these issues for significant motor function restoration in SMA patients.

Introduction

Spinal Muscular Atrophy (SMA) is a genetic disease obtained mainly through autosomal recessive inheritance, in which individuals lack both copies of the survival motor neuron one (SMN1) gene (*Spinal Muscular Atrophy | National Institute of Neurological Disorders and Stroke*, 2023). Anterior motor neurons are degenerated without this gene's expression, resulting in muscular atrophy that leads to paralysis and death (*Inhibition of Apoptosis Blocks Human Motor Neuron Cell Death in a Stem Cell Model of Spinal Muscular Atrophy*, 2012). SMN1 encodes survival motor neuron (SMN) protein, which joins together with other proteins to form the SMN complex (Thomas, 2018). This molecule helps synthesize small uridine-rich nuclear ribonuclear proteins (U snRNPs) that are pivotal for the creation of spliceosomes, which splice pre-mRNA during RNA processing. Though SMN protein is found in all cells, its absence profoundly affects motor neurons for an unclarified reason.There are four types of SMA (I-IV) based on the severity of the condition, which is measured by the age of onset and physical milestones achieved (*Spinal Muscular Atrophy (SMA*), 2022). A C to T nucleotide transition (a C located at position 6 in exon 7 in SMN1, whereas a T is located at the same position in SMN2) results in exon 7 of the SMN protein transcript being removed during RNA splicing, and thus a nonfunctional SMN protein is produced (Wang & Connolly, 2016). However, about 10% of the pre-mRNA produced by SMN2's transcription is properly spliced into functional SMN protein. This is why individuals with SMA are able to still undergo embryonic development, but cannot maintain their spinal cord motor neuron health.

Current treatments of SMA have been approved very recently, and include nusinersen (Spinraza™), onasemnogene abeparovec-xioi (Zolgensma ™), and orally-administered drug risdiplam (Evrysdi®). The drug Spinraza involves the injection of antisense oligonucleotides specific to SMN2 transcripts (*How SPINRAZA® (Nusinersen) Works | HCP*, 2024). These synthetic, single-stranded pieces of RNA temporarily bind to SMN2 pre-mRNA downstream of exon 7, where they promote the inclusion of exon 7 in the mature transcript by preventing certain heterogeneous nuclear ribonucleoproteins (hnRNPs), a class of RNA-binding proteins involved in RNA processing and other aspects of protein synthesis and regulation, from associating with the precursor RNA (*How SPINRAZA® (Nusinersen) Works | HCP*, 2024; Rossor, et al., 2018; *The HnRNP Family: Insights Into Their Role in Health and Disease*, 2016). These injections take place four times a year (Pope, 2023). The treatment of Zolgensma uses a single injection of the AAV9 vector (a virus used to carry DNA into host cells in gene therapies), in which the DNA of the virus has been removed and replaced by a functional SMN gene (*How Gene Therapy Works | ZOLGENSMA® (Onasemnogene Abeparvovec-Xioi)*, 2023; *Vector*, 2024). The vector spreads throughout the body, inserting the SMN gene into motor neurons and other cells, thus allowing for them to produce enough SMN protein for survival. However, this therapy is only available for children up to two years of age. Lastly, Evrysdi is an oral medication that modifies SMN2 splicing by allowing exon 7 inclusion in final transcripts, which must be taken daily (*Risdiplam, the First Approved Small Molecule Splicing Modifier Drug as a Blueprint for Future Transformative Medicines*, 2021).

These therapies focus on improving SMN protein production, but are unable to reverse motor neuron death in those with SMA. This is a major issue for older patients who have not received treatment, as their motor neurons have undergone significant neurodegeneration, resulting in their recovery being marginal. Moreover, while newborn screening for SMA in the US has become more common in recent years, thus giving families ample time to minimize the pathology of SMA by giving them these therapies at a very young age, there is a significant financial barrier present for all these treatments. As of 2023, Zolgensma had a cost of $2.1 million dollars (though they do offer a five-year payment plan at $425,000 a year), while Spinraza costs $750,000 for the first year, and $350,000 per year afterwards (Pope, 2023). Evrysdi costs $13,504 for 80 mL (equivalent to 80 mg), with the daily dosage for each individual varying based on their weight and age, but maximizing out at 5 mg daily (*Evrysdi Prices, Coupons, Copay & Patient Assistance*, 2024; *Find The Evrysdi® (Risdiplam) Dosing Information | Official Healthcare Professional Site*, n.d.). The maximum cost is around $340,000 a year, thus being lower than Spinraza (*Royalty Boosts Struggling PTC With Additional $1B for Royalties on Roche's Evrysdi*, 2023). These costs may prevent patients from receiving treatment as soon as possible, forcing them to undergo motor function loss that cannot be retained at a later age. Additionally, there are also a small number of people with SMA who received placebos during the clinical trials for currently approved SMA drugs and therapies. This population has not been able to benefit as much from treatment at a later age, and also deserves to be helped. For these individuals, a solution would be the development of a cell replacement therapy for SMA. Specifically, stem cells show promise in replacing motor neurons in the spinal cord, and thus could be used to develop a treatment that reverses the neurodegeneration in SMA patients. They can also provide neuroprotective factors to existing motor neurons, hindering disease progression (Corti et al., 2010). Even with the immense potential for a stem cell driven neuroregenerative therapy for SMA, no human treatment has been developed, with there currently being no approved stem cell treatments for other neurodegenerative diseases as well. Many challenges to the development of both a safe and effective stem cell treatment, especially in the context of SMA, need to be overcome before this happens.

There are many "stem cell clinics" in the U.S and across the globe, offering treatment for various disorders but have little to no proof of their efficacy and have resulted in adverse side effects (Kuriyan et al., 2017). For instance, intrathecal stem cell infusions into the spinal cord by stem cell clinics resulted in the formation of unwarranted glial proliferative lesions in Argentina, China, and Mexico. Regardless, there are various legitimate clinical trials where stem cells are being tested to treat these conditions. A journal article published in August 2023 in Stem Cells Translational Medicine reviewed the clinical trials using neural stem cells to treat various neurodegenerative diseases around the world, though these did not include SMA. Interestingly, amyotrophic lateral sclerosis (ALS), a disease with similar

pathology to SMA, had 32 clinical trials listed, and there was even one that was temporally successful (Fan et al., 2023). ALS systematically affects two or more levels of the motor neuron networks (lower, corticospinal, & prefrontal) and neurons in the brain, leading to the disease impacting multiple regions of the body (Armon & Lorenzo, 2024). This means ALS can also affect lower motor neurons (LMNs), including anterior motor neurons. Though there are different types of ALS depending on which motor neurons are affected, those that involve the loss of LMNs lead to atrophy and progressive muscle weakness, just like in SMA. Other shared features between the two conditions include defects in the pre- and postsynaptic areas of neuromuscular junctions (NMJs), metabolic disruptions affecting the whole body, and the atypical excitability of motor neurons (Bowerman et al., 2018). The actual method of neuron degeneration in ALS is not precisely defined, though genetic mutations, neuroinflammation, abnormal synaptic function and other causes have been attributed to the condition (Brotman, 2024). A phase one clinical trial in Italy for ALS showed it is possible for stem cell transplantation to have a positive effect on motor function (Mazzini et al., 2015). Patients with ALS were injected with human fetal neural stem cells (hNSCs) in the anterior horn region of their spinal cords. Fetal stem cells were obtained from natural miscarriages, resulting in the elimination of ethical concerns. Disease progression halted for 18 months because of treatment, and two of the six patients experienced increased ambulation (walking) scores. The exact cause for this improvement could not be verified, though a likely reason was thought to be the release of various molecules by the NSCs that produced anti-inflammatory effects. Understanding the distinct pathologies in SMA and ALS would help stem cell therapy treatments be better curated for both conditions. Moreover, it might shed light on if there is any application for how a stem cell therapy for ALS may be applied to help those with SMA. Regardless, this study shows that stem cells are able to ameliorate neurodegeneration in the human spinal cord, which is crucial for the development of a stem cell treatment for SMA.

In the U.S, the Food and Drug administration has reclassified manipulated stem cells as drugs since 2017, rendering them subject to their approval (Hiltzik, 2021). However, they gave stem cell clinics three and a half years to adjust to these regulations, thus using limited enforcement of their new policy until May 2021. Unfortunately, there was little compliance to FDA regulations, and instead, these clinics proliferated to a number greater than before. In November 2021, there were 1,480 businesses identified to be operating 2,754 clinics nationwide in a study published by Leigh Turner in the journal *Cell Stem Cell*. He mentions that the FDA does not have enough inspectors for such a large quantity of companies, and stem cell treatments have become common, being sold by chiropractors, sports medicine clinics, and more. U.S. clinical trials for SMA do not involve stem cell transplantation. Current tested therapies are combination therapies, studying the effects of known SMA treatments together and how they work with another drug: apitegromab (*Spinal Muscular Atrophy: The Past, Present, and Future of Diagnosis and Treatment*, 2023). It is an inhibitor of inactive myostatin, a growth factor in the TGFβ family (*SRK-015 - Therapy for Spinal Muscular Atrophy (SMA)*, 2024). Primarily expressed in skeletal muscle cells, it regulates muscle mass through working with other hormones and growth factors, and prevents muscle growth. Increased muscle mass and strength were observed in vertebrates who lacked the myostatin gene, with there being little effect on their health otherwise. While this shows immense promise for preventing muscle atrophy in individuals with SMA, it still does not solve the lack of synaptic activity to some of those muscles caused by motor neuron death, meaning motor function recovery will still be limited. A stem cell transplantation therapy, on the other hand, has the potential to give back a fuller range of motion depending on the amount of motor neurons that are able to successfully integrate and survive in the spinal cord.

A study that concluded in 2016, located in Minnesota, tested the safety of mesenchymal stem cells in an ALS treatment (Staff et al., 2016). Stem cells from each patient were separated from adipose (fat) tissue and differentiated into ones that excited neuron growth factors. They were then transplanted into the patient's cerebral spinal fluid and along protective membranes around the spinal cord. While there were no negative side effects found in this trial, the treatment was unable to prevent ALS progression in any of the twenty-seven patients. Regardless, this study shows that stem cells can be used as a safe treatment for neurodegenerative disease.

The creation of a stem cell transplantation therapy is a worthy endeavor for patients with SMA, who, for financial or other reasons, did not receive treatment at a young age. This could give them back motor function that is

not yet possible with current SMA treatments. Furthermore, such a therapy has the potential to be applied to other motor neuron diseases, including ALS.

Methodology

The objective of this study was to review progress in the development of a stem cell therapy for SMA, explaining the biological phenomenon behind its effectiveness and current challenges in application. Research was conducted in the form of a literature review, with both primary research studies and explanatory articles being discussed. The paper first goes over stem cells in general, explaining their general applications and ethical issues, before giving an overview of the spinal cord and its microenvironment. This is used to give background information before deeply analyzing stem cell treatment experiments for SMA using animal models. The mechanisms of amelioration, translational issues, and safety of these transplants are reviewed, as well as their implication for future research in developing a regenerative stem cell therapy for SMA. As only online resources were used in this study, there are no ethical considerations to observe.

Spinal Cord Structure

The spine is composed of the spinal cord, made up of tissue, nerves, and other cells in a fragile, cylindrical band, and the vertebral column, the bone layer protecting the spinal cord from injury (*Spinal Cord: Function, Anatomy and Structure*, 2021). The spinal cord has three main parts and three membranes (composing the meninges) surrounding it. It is broken down into the cervical, thoracic, and lumbar sections, corresponding to the part in one's neck, chest, and lower back, respectively. In terms of its membranes, the outermost layer is dura mater, a durable membrane functioning to prevent physical damage. The middle layer is arachnoid mater, a thin layer from which connective tissue extensions attach to pia mater (*Meninges: What They Are & Function*, 2022). Finally, there is the pia mater, the innermost layer, which tightly wraps around both the spinal cord and brain. The subarachnoid/intrathecal space lies between the arachnoid and pia mater and contains cerebrospinal fluid (CSF), clear liquid that circulates nutrients throughout the central nervous system (CNS) while removing waste (*Meninges: What They Are & Function*, 2022; Neuman & Maiuri, n.d; National Cancer Institute, n.d.).

Neuron cell bodies in the spinal cord collectively form gray matter in an "H" shape around the intermediate canal (Feng et al., 2022). Gray matter can be broken down by sections into posterior horns, a median zone, and anterior horns. Sensory information is transmitted to neurons within the posterior/dorsal region through dorsal roots, whereas motor signals are transmitted from motor neuron bodies within the anterior/ventral region to innervated muscles through ventral roots (Purves et al., 2001). The ventral and dorsal roots eventually connect through interneurons to form spinal nerves, that, after splitting into dorsal and ventral rami, each of which contain axons from motor and sensory neurons, extend to the periphery of the body (Kaiser et al., 2024). White matter surrounds the gray matter of the spinal cord, and consists mostly of glial cells, axons, and blood vessels (Feng et al., 2022). The brain and spinal cord are connected by vertical fiber tracts (columns) in the white matter. SMA pathology is primarily caused by motor neuron degeneration within the anterior horns of the spinal cord, and thus, understanding its structure is critical for comprehending the proper migration of stem cells through it during transplantation.

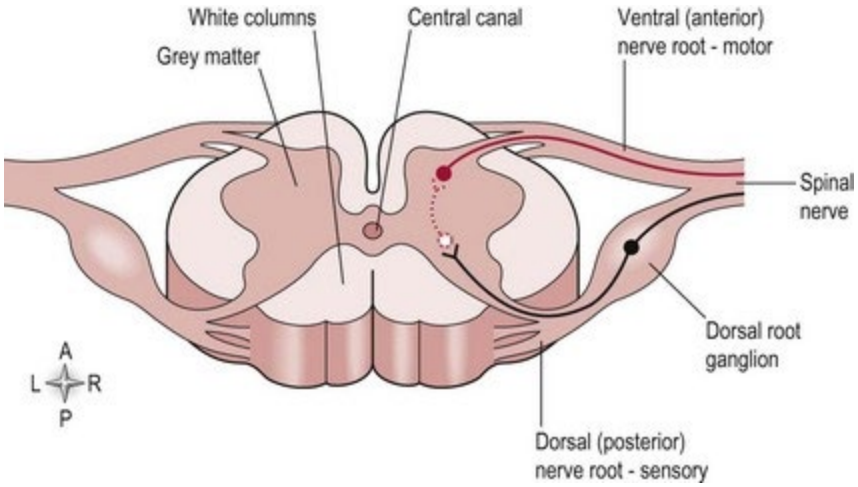

Figure 1. Spinal Cord Cross-Section**.** Source: *The Spinal Cord and Spinal Reflexes*, n.d. Description: Diagram detailing the location of gray matter, white matter columns, the central canal, and nerve roots in the spinal cord. Compass indicates the perspective of the cross-section: A stands for anterior, P stands for posterior, L stands for left, R stands for right.

Stem Cells

Stem cells are self-renewing with the capability of differentiating into multiple types of cells (*Stem Cell Sources, Types, and Uses in Research*, 2023). Their potency, or differentiation potential, can be classified as totipotent (can become all types), pluripotent (nearly all types), multipotent (closely-related types), oligopotent (few types), and unipotent (one type). They can be found throughout the body for repair after injury (*Stem Cells: What They Are and What They Do, 2024*). However, this group of stem cells, adult stem cells, generally have more limited potency, only differentiating into cell types associated with the tissue in which they reside, than the other main sources, being embryonic and induced pluripotent stem cells (ESCs and iPSCs respectively). ESCs can turn into all cell types within the developing organism, and iPSCs, adult stem cells genetically reprogrammed into a pluripotent state, have similar differentiation potential. Progenitor cells are also of note, as these direct stem cell descendants also differentiate into fixed cell types (Bhartiya, 2015). These cells' ability to become specific types of cells allows them to be utilized as regenerative medicine, where they can replace damaged or diseased tissue through transplantation (*Stem Cells: What They Are and What They Do, 2024*). As discussed earlier, this could be invaluable for SMA patients to reverse motor neuron degeneration.

However, there are ethical and technical issues that need to be considered when utilizing stem cells, which serve as general challenges to creating a stem cell therapy. The extraction of ESCs from an embryo destroys it, raising concerns due to the various views of its moral status (Park et al., 2024). For instance, Catholicism commonly deems life as beginning once an egg is fertilized, and thus does not see embryonic stem cell research as justifiable. On the other hand, while iPSCs can avoid these ethical concerns, they have more technical issues instead. Along with the inefficiency of reprogramming these cells, compared to embryonic stem cells, they are not as potent, have more transcription errors, have a lower growth rate (*Stem Cells: What They Are and What They Do*, 2024; *Stem Cell Sources, Types, and Uses in Research*, 2023). Adult stem cells likely have more abnormalities due to environmental hazard exposure or replication errors in addition to their limited potency (*Stem Cells: What They Are and What They Do*, 2024).

Neural Stem Cells - Spinal Cord Transplantation

Regardless, various stem cell treatment models in SMA mice have shown success, these cells successfully integrating into the host spinal cord environment and ameliorating SMA symptoms. These experiments, done by Corti et al. (2008, 2009, 2010, 2012), involve the transplantation of neural stem cells (NSCs) or stem-cell derived motor neurons into mice spinal cords afflicted with SMA. NSCs are multipotent, capable of differentiating into neurons, astrocytes, and oligodendrocytes, the three main cell types making up the CNS (Okano, 2010). In fact, they are responsible for CNS development, starting in a stage known as neural induction. Well-established NSC subpopulations have been generated by a number of differentiation procedures from PSC sources, including human embryonic stem cells (hESCs) and iPSCs (De Gioia et al., 2020). Generally, they are developed by first creating embryoid bodies, three-dimensional clusters of PSCs, that are then placed into a medium with specific growth factors, such as fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and others (De Gioia et al., 2020; Lin et Chen, 2014). Nestin, a few members of the Sox family, and other molecules are used as selective markers for NSCs and neural progenitor cells (NPCs) within the CNS (Okano, 2010). These markers encode proteins making certain selective agents ineffective against a specific cell type, allowing cell distinguishment and selection when transforming a culture into NSCs (Bhatia et Dahiya., 2015). Fluorescence-activated and magnetic bead cell sorting can be used to purify a differentiating NSC culture further based on their specific surface markers. For instance, p75-NTR antigen expression was used for magnetic cell separation of motor neurons from NSCs for transplantation into Spinal Muscular Atrophy with Respiratory Distress Type 1 (SMARD1) mice (De Gioia et al,. 2020; Corti et al., 2009). Unlike SMA, SMARD1 is caused by nonfunctional *IGHMBP2* genes that encode Ig micro-binding protein 2 (*Ighmbp2*). Similar to SMN protein, however, is *Ighmbp2* expressed throughout the body, but selectively impacts motor neurons for poorly researched reasons (Corti et al., 2009).

NSC transplantation has been shown to administer a therapeutic effect against neurodegeneration in a variety of ways, including through the release of neurotrophic factors, cell replacement, and more (De Gioia et al., 2020).

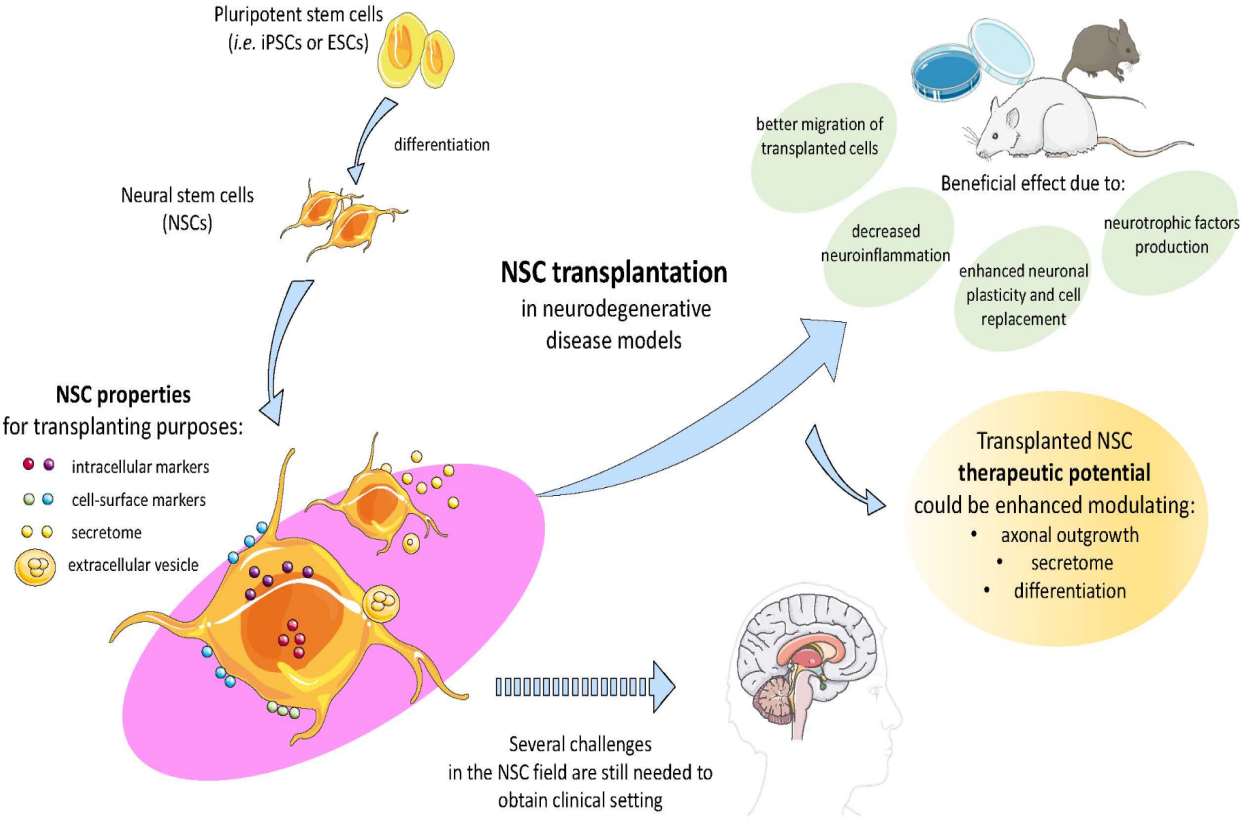

Figure 2. NSC Transplantation in Neurodegenerative Disease Models**.** Source: De Gioia et al., 2020**.** Description: PSCs are differentiated into NSCs with specific properties (have intracellular markers, cell-surface markers, a secretome, and extracellular vesicles) that make them appropriate for CNS transplantation. Challenges, such as proper migration and integration of NSCs, block current clinical application. Amelioration of disease symptoms can be attributed to a variety of causes, and could be furthered by altering the axonal outgrowth of exogenous neurons, their secretomes, or differentiation protocols.

Advantageous effects of NSC transplantation are dependent on the grafted cells' ability to travel to and throughout afflicted areas (De Gioia et al., 2020). Intrathecal spinal cord injection of NSCs has been used in stem cell experiments of SMA mice models (Corti et al., 2008; Corti et al., 2010). Studies have shown that these transplanted cells travel to the spinal cord and differentiate into glial and neuronal subpopulations, a key component of creating a minimally intrusive stem cell therapy (Corti et al., 2008; Corti et al., 2010). NSCs possess chemokine receptors that direct them towards damaged tissue. Unfortunately, those same signals that NSCs respond to are expressed by themselves, which has been shown to create clumping (De Gioia et al., 2020). After reaching the designated area, NSCs must be able to survive and integrate into host circuitries for motor function recovery.

The secretome, all the proteins and other factors excreted by a cell, of NSCs has been shown to be made up of neuroregulatory molecules that play roles in migration, differentiation, and neuroprotection of nerves, as well as neurotrophic factors (Davey, 2020; De Gioia et al., 2020). As a result of these factors, when together, endogenous neurons experience decreased cell death and increased axonal growth, while the transplanted NSCs experience increased integration and survival (De Gioia et al., 2020). In fact, overexpression of neurotrophic molecules by NSCs, including GDNF, BDNF, NT-3, and NGF, has been shown to increase NSCs' neuroprotective potential and

sustainability further in neurological disease models (De Gioia et al., 2020). For instance, in the spinal cords of ALS model mice, NSCs overexpressing GDNF efficiently infiltrated and integrated into the diseased host environment. The secretome may also contribute to a reduction in neuroinflammation, which is present in both ALS and SMA, and has been indicated to play a major role in the beneficial effect of NSC transplantation. Indeed, prolonged inflammation in the spinal cord can accelerate neurodegeneration through neuron and oligodendrocyte death while also hindering axon growth (Lee et al., 2022). There is increasing support for the idea that inflammation can cause protein aggregation within neurological diseases, thus creating a more neurotoxic environment (Zhang et al., 2023).

While support to endogenous motor neurons can be valuable for an SMA patient, of greater beneficial potential is the ability to create exogenous motor neurons that then integrate into the spinal cord. Only a small proportion of transplanted NSCs have been shown to develop a motor neuron phenotype in SMA mice (Corti et al., 2008; Corti et al., 2010). The disproportionality of stem cells to differentiated neurons can be attributed to the fact that signals within the adult CNS gliogenesis over neurogenesis (Gioia et al., 2020). Genetically modifying NSCs can be used to drive their differentiation towards a motor neuron phenotype. Though this did show increased success in an SMA mice model, overall motor neuron differentiation was still limited (Corti et al., 2010). Alternatively, motor neurons can be cultured in vitro and then directly injected, a method that has shown success in terms of disease amelioration in SMA mice models (Corti et al., 2009; Corti et al., 2012). Human-iPSC derived motor neurons injected into SMA mice models were also able to produce neurotrophic factors, contributing to endogenous motor neuron support while increasing the mean number of motor neurons in the ventral horns of their spinal cords (Cort et al., 2012).

Microenvironment of the Spinal Cord in SMA

The microenvironment of the spinal cord is affected by various cell types, including neurons, glial cells, immune cells, and fibroblasts (Feng et al., 2022). An appropriate microenvironment is needed for neuron regeneration, as it controls the growth, proliferation, and integration of cells that are transplanted within the column. The largest population of cells within the CNS, glial cells, including microglia, astrocytes, and oligodendroglia, and their progenitors, surround and provide trophic support to neurons (Abati et al., 2020). Microglia are responsible for destroying dysfunctional cells and foreign invaders. They secrete cytokines, molecules facilitating immune system activation, with their amount varying based upon stimuli from the microenvironment (*What are Cytokines? Types & Function*, 2023). Intrinsic signals influence microglial phenotype, turning them into either a pro-inflammatory or anti-inflammatory variation (Abati et al., 2020). Though these types are commonly referred to as M1 (pro-inflammatory) and M2 (anti-inflammatory), modern transcriptome profiling has revealed that their changes based on stimuli are much more complex to be classified into two subgroups (Matejuk et Ransohoff, 2020). After neuron loss, they induce “reactive microgliosis,” producing an inflammatory response with the help of other types of cells (Abati et al., 2020). Astrocytes have a large variety of neuro-supportive and regulatory functions, including nourishment transferring to nerve cells from the bloodstream, controlling the differentiation and maturation of neurons, and managing synaptic activity. Immune T-cells (white blood cells) and neurons majorly influence the actions of astrocytes. Oligodendroglia wrap neurons in myelin, significantly increasing action potential conduction, and thus the rate of transmission of neuron signals (Purves et al., 2001). They have also been shown to be neurotrophic through delivering growth factors such as GDNF or metabolic substrates like lactate (Abati et al., 2020). Lastly, Schwann cells wrap myelin around peripheral nerve cell axons and also sustain the extracellular matrix (ECM) by releasing certain proteins (Purves et al., 2001; Abati et al., 2020).

These glial cells play key roles in maintaining a healthy CNS environment, but certain types have been shown to become dysfunctional in SMA, contributing to pathogenesis and creating issues in the creation of a stem cell therapy (Abati et al., 2020; Rigby et al., 2022).

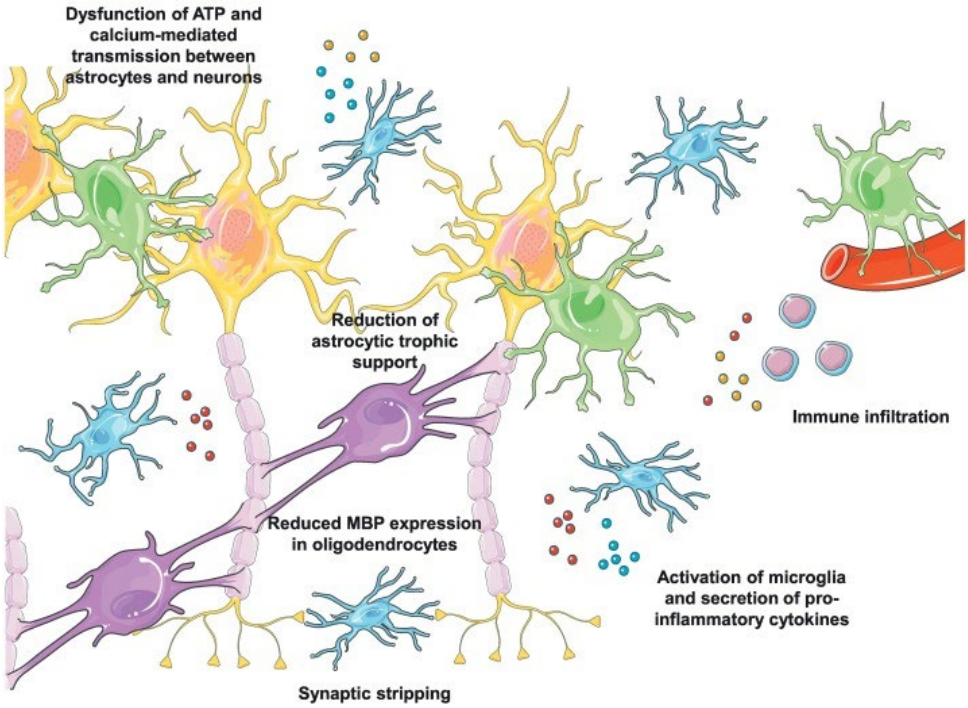

Figure 3. SMA Pathology in the CNS. Source: Abati et al., 2020**.** Description: Various glial processes contribute to SMA pathology within the CNS. Due to incorrect ATP and calcium mediated signaling, astrocytes and neurons do not properly communicate with each other. Additionally, defects in astrocyte structure create a lack of trophic support for motor neurons. Proinflammatory cytokine production and apoptotic cascade activation are caused by elevated levels of M1 microglia. Microglia also contribute to synaptic stripping, which leads to synaptic loss. Further immune cell entrance into the CNS is caused by this neurotoxic environment, contributing to more neuroinflammation and pro-inflammatory activation of glial cells.

Areas of the spinal cord afflicted with motor neuron degeneration were also shown to be correlated to gliosis in every type of human SMA (Abati et al., 2020). These reactive glial cell proliferations block neuron regeneration and differentiation through creating a physical barrier to regenerating axons (Rigby et al., 2020). Indeed, glial bundles are found in degenerated motor neuron areas with activated glial cells, being a critical feature of SMA (Abati et al., 2020). The diseased spinal cord microenvironment in SMA contributes to motor neuron loss, dysfunction, and may limit the survival of exogenous ones transplanted into the anterior horn.

Fortunately, the use of current SMA treatments could create a more favorable environment for cell engraftment. Specifically, restoration of SMN protein production in glial cells, now possible due to approved therapies, can normalize their function, and thus increase their capabilities for motor neuron support. For instance, astrocytes have also been observed as structurally deficient and reactive long before the death of motor neurons in SMA pathology, underlining the fact their dysfunction may be resulted solely from a lack of SMN rather than the CNS microenvironment (Abati et al., 2020). Moreover, a study by Rindt et al. (2015) showed that restoring normal SMN protein levels to just astrocytes in SMA mice models through a viral vector method ameliorated disease pathology. Once SMNΔ7 mice, a common utilized model of SMA, received the vector, their glial activation marker levels, including IL-1β, IL-6 and TNFα, went towards those observed by normal mice, implying that SMN restoration in astrocytes reduces glial activation and thus neuroinflammation. This may reduce gliosis in SMA spinal cord and thus decrease potential barriers to transplanted cell integration. Motor neurons themselves also benefited, as shown by the increased amount of vGLUT1+ synapses on their somata compared to untreated littermates. These synapses are responsible for transmitting motor and location information (proprioception) from sensory neurons, and are steadily lost in SMNΔ7 mice

(Melinosky, 2024; Rindt et al., 2015). Furthermore, this treatment also resulted in abnormality correction of neuromuscular junctions (NMJs), as well as less denervated NMJs, in observed muscles, namely the splenius capitis and longissimus capitis (Rind et al., 2015). However, it is important to note that the amount of anterior motor neurons between untreated and treated mice did not differ, and increased axon growth as a result of the corrected astrocytes was minimal. On the other hand, when SMA animal models are treated with SMN vectors expressed throughout the body, motor neuron levels are near that of wild type animals. This difference can be attributed to the fact that the motor neurons still lack SMN protein levels and undergo degeneration, and also implies that the corrected astrocytes still created the proper microenvironment for SMA motor neurons to remain functional. With this in mind, it could be beneficial to combine an SMN production therapy with stem cell treatment, though further research is needed to determine an enhanced effect.

Muscle Innervation - Final Step for Restored Motor Function

Of course, motor neuron survival within the host spinal cord is not only part of what is needed for restored motor function (De Gioia et al., 2020). Differentiated neurons from transplanted NSCs must join to existing circuitries and have long axonal extensions, a challenge when in an adult CNS afflicted with disease. Neuromuscular junctions, peripheral axons, and Schwann cells have all been implicated to be dysfunctional in SMA pathology (Abati et al., 2020). In particular, Schwann cells in SMA have been found to facilitate the incorrect myelination of axons, leaving them susceptible to degeneration and thus creating a dysfunctional connection between the brain and body (Abati et al., 2020; Stenudd et al., 2014). Fortunately, similar to astrocytes, correcting SMN protein production with Schwann cells has been shown to reverse negative effects, such as by improving NMJ function and fixing myelination abnormalities, in mice. It is important to note, however, that current SMA treatments differ in their ability to affect the peripheral nervous system (PNS). Spinraza is an intrathecal therapy, and thus unable to reach the PNS, whereas risdiplam can increase SMN protein levels in peripheral tissue and Zolgesma affects the whole body (Abati et al., 2020; Poirier et al., 2018).

Nevertheless, axonal growth in the adult CNS is still far below the level found in its development, which may be associated with its limited regenerative capacity (De Gioia et al., 2020). Genetically modifying NSCs and NSC-derived cells to overexpress factors associated with axonal extension, treating cells with substances that increase axonal growth before transplantation, and altering the host milieu to make it more receptive to axonal elongation are all strategies that have been tested to resolve this issue.

For instance, host environment alteration and substance use before transplantation were used to facilitate extended axonal projections from motor neurons in *nmd* mice, a SMARD1 model (Corti et al., 2009). Motor neurons were generated from an NSC subgroup derived from murine embryos that expressed Lewis X. Cells, after differentiation protocol, expressed HOXC6 and HOXC8, which are markers of spinal cord motor neurons. As previously stated, motor neurons were selected for using magnetic cell separation. These cells were also altered to express GFP under the HB9 promoter of motor neurons. Treatment groups included the following: *nmd*-trans, only receiving motor neuron transplantation; *nmd*-veh, only receiving mock transplantation; *nmd*-trans+drugs, which had both drug therapy and motor neuron transplantation; *nmd*-veh+drugs, just receiving drug therapy; and a wt group containing mice unaffected by SMA.

The drug therapy for axonal growth promotion consisted of hypodermic injections of rolipram, a substance utilized for overcoming inhibitory signals on axon elongation produced by myelin proteins, two days after transplantation, as well as injections of GNDF in limb muscles, used in an attempt to increase attraction of transplanted neurons to their innervation targets, starting the day of transplantation. Motor neurons that were going to be injected into drug therapy mice were also put into a dbcAMP solution for enhanced axonal elongation and survival. 20,00 cells were injected in total between the horns of cervical and lumbar sections of the spinal cord. At eight weeks after transplantation, *nmd*-trans+drugs and *nmd*-trans mice performed significantly better than groups that received mock motor neuron transplantation (*nmd*-veh and *nmd*-veh+drugs), though not as well as the wt mice. Additionally, *nmd*-

trans+drugs mice performed better than *nmd*-trans mice. Along with an observed increase in body mass in comparison to vehicle groups, these results indicated that motor neuron transplantation ameliorated the SMARD1 phenotype, while also supporting the possibility of an enhanced therapeutic effect due to axonal growth modulation.

When comparing *nmd*-trans and *nmd*-trans+drug spinal cords at the end stage of disease, both were found to have an abundance of transplanted motor neurons (as shown by their expression of GFP). Though the percentage of engrafted neurons in between groups was not statistically significant, HB9-GFP neuron axons extended more into white matter in *nmd*-trans+drugs than those in *nmd*-trans, and also had more axons within ventral roots of the spinal cord, thus indicating an increased connection to the PNS. Moreover, GFP positive axons in skeletal muscles were evident only in the combined therapy group, and there was also acetylcholine receptor clustering that indicated NMJ formation (Corti et al, 2009; Bai and Zhang, 2021). The structure of older and recently formed NMJs were shown to be alike.

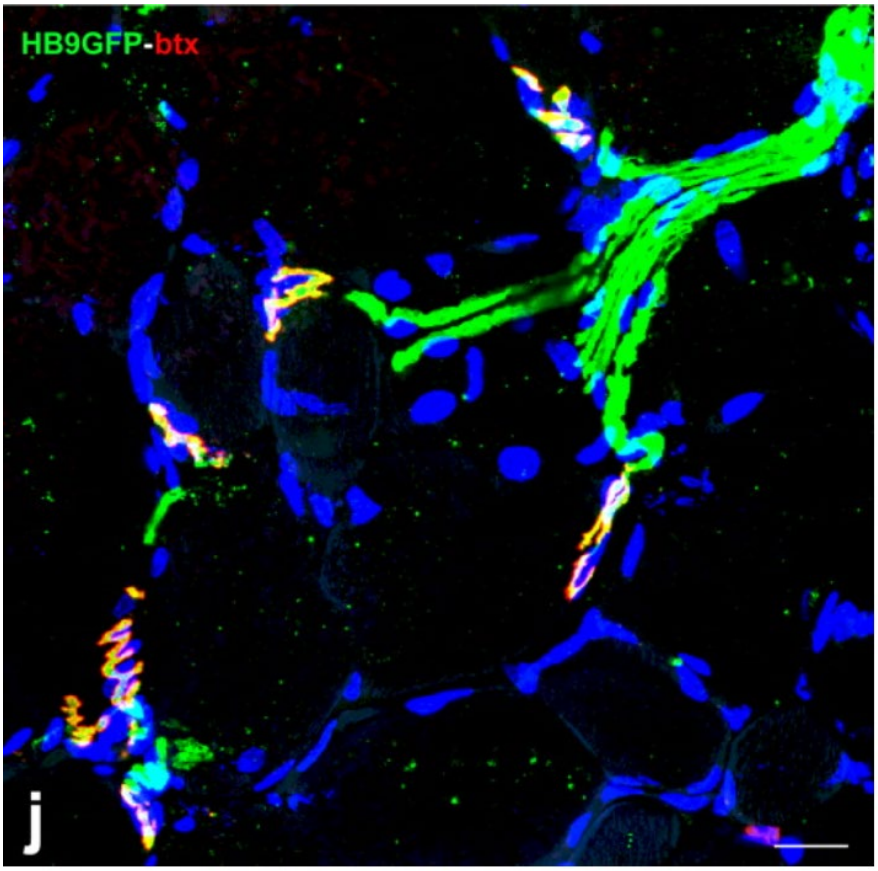

Figure 4. In Vivo NMJ Formation From Transplanted Motor Neuron Axons**.** Source: Corti et al., 2009**.** Description: (j) Axons of HB9-GFP motor neurons (in green) extended into skeletal muscle tissue and formed NMJs, as shown by rhodamine-conjugated bungarotoxin (bright orange).

This experiment showed that drug therapy can be effective in environmental modulation to enhance axonal outgrowth. Further research could elucidate if there are strategies utilized alongside rolipram for increased therapeutic effect, and will be needed for appropriate dosage if ever used for clinical application (De Gioia et al., 2020) .

Stem Cell Therapy Models for SMA - Selected Experiments

Spinal Cord-Derived NSC Transplantation

The injection of spinal cord-derived NSCs into SMA mice by Corti et al. (2008) likely represents the first instance of stem cell transplantation to determine the possibility of a restorative effect. 20,000 ALDHhiside scatterlo (ALDHhiSSClo) cells, a self-renewing NSC subpopulation, were intrathecally transplanted at postnatal day one. They were also modified for HB9-GFP expression. Controls for the experiment included transgenic SMA mice that were injected with an ineffectual vehicle and wild type (wt) mice.

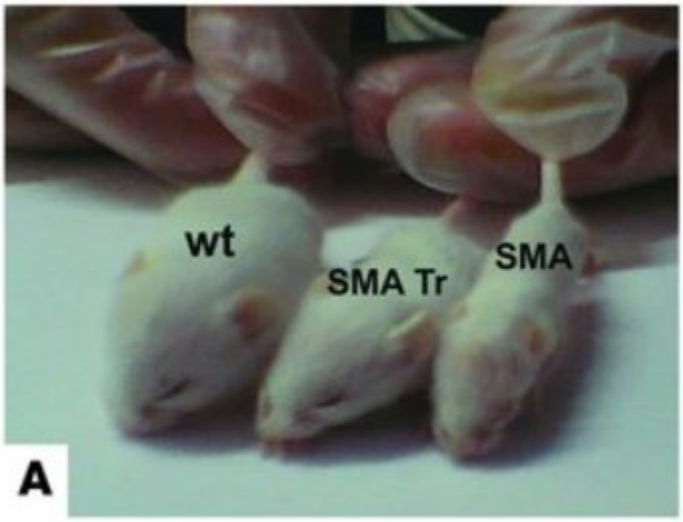

Figure 5. NSC Transplantation Visibly Improves SMA Phenotype in Mice. Source: Corti et al., 2008. Description: (A) Photograph of a mouse unaffected by SMA (wt), one with SMA that received NSC treatment (SMA Tr), and another with regular SMA pathology.

Along with improved physical appearance, mice with NSC treatment had a notable increase in survival time and weight compared to their untreated counterparts. They also performed better on the grip assay test, in which the time that mice can use their forelimbs to hold up their weight on a metal rail is measured. At 12 days old, the WT mice could hold themselves up for ten seconds, the treated mice exhibited decent performance at three seconds, and untreated mice could not grip.

ALDHhiSSClo cells were able to effectively migrate into spinal cord parenchyma after delivery to the CSF, first laterally clipping to the meninges before infiltrating the pia mater and then going into the gray and white matter of the spinal cord. Once the treated mice had reached the end stage of their disease, transplanted motor neurons were able to be detected due to their HB9-GFP–positivity. In both the cervical and lumbar enlargements, there were GFP-positive cells, showing differentiation into neural phenotypes. Detectable NSC-derived motor neurons had long neurites in the gray matter and were smaller than host motor neurons, which is anticipated of more newly developed cells. However, based on choline acetyltransferase (ChAT) and HB9 expression (both being markers of a true motor neuron phenotype), only about 3.5% of cHAT motor neurons were donor-derived.

The axons of these motor neurons were able to protrude into white matter. A small number of motor neurons also had processes that entered the ventral roots (27 ± 11 GFP-positive axons on average per animal) that signified the possibility of axon integration into the periphery. The scarce amount of donor-derived motor neurons and restricted axon growth signified that motor neuron replacement was not the main source of disease amelioration. To investigate

further, these researchers looked at the total number and diameter of motor neurons within the lumbar sections of the spinal cords of the treatment groups. They found that there was an approximately 39% reduction in motor neuron number when comparing wt mice to untreated SMA mice, but only an approximately 24% reduction in number when comparing wt mice to treated SMA mice. Treated SMA mice had motor neurons with a greater diameter than the control SMA mice and less than those of the wt mice. The treated group also had significant improvement in myofiber (elongated cells that make up muscle tissue) quantity, diameter, and area compared to the untreated group. Similarly, NMJ diameter was also greater than the untreated group, thus showing motor axon terminal protection (Corti et al., 2008; Cretoiu et al., 2018). These factors imply that NSC treatment is able to affect the function of whole motor units.

When comparing the endogenous motor neurons of SMA mice from the treated and untreated groups, 42 genes were found to differ in expression levels, many of which were associated with proteins facilitating RNA processing and translation. Treatment was shown to bring gene expression towards that of wt mice. There was another set of "early response genes", however, that did not follow this trend, most likely indicating SMA motor neuron response to the growth factors secreted by NSCs. To further analyze this treatment's therapeutic effect, a co-culture system, where the top layer was a porous membrane, allowing solely soluble factors to pass through, populated with ALDHhiSSClo NSCs, and the bottom layer had primary spinal motoneurons (PMNs) from SMA mice, was compared to untreated PMNs from SMA mice. Mean axon length and growth cone size were shown to increase compared to the untreated group. The level of cytokine and neurotrophin secretion was then compared between the cell supernatants of undifferentiated NSCs, primed NSCs, astrocyte derived NSCs, and primary fibroblasts. Primed NSCs were shown to secrete high levels of GDNF, BDNF, NT3, and TGF-a. Higher VEGF secretion was observed by all NSC-derived cells in comparison to fibroblasts. VEGF has a role comparable to that of angiogenesis factors, which are significant players in both neurogenesis and neuroprotection. These neurotrophins were able to impact axon growth as shown by the coculture.

Overall, this experiment created the amelioration of disease phenotype based mainly on neurotrophic factor promotion, though there was also small amount of neurogenesis and subsequent motor neuron integration. However, the use of NSCs derived from the spinal cord creates limits for clinical translation due to their scarcity within an adult CNS, and results in other ethical and technical issues (Corti et al., 2010). Using PSC sources (ESCs or iPSCs) would create a potentially limitless and better characterized source of NSCs for transplantation, though a therapeutic effect would have to be proven and compared to the spinal-cord-derived NSCs. This is what Corti et al. successfully tested in their next experiment.

ESC-Derived NSC Transplantation

Wild-type and OSG ESCs were each differentiated into NSC sources before being transplanted into different groups of SMA mice at one day of age (Corti et al., 2010). OSG ESCs were genetically modified so that neuron differentiation could be enhanced through negative selection for undifferentiated stem cells, and both stem cell sources expressed the GFP gene. Fibroblasts were also injected to a group of SMA mice to measure a comparative effect, and there was an additional control group that received no treatment.

Murine ESCs were primed into NSCs by being cultured in a neurobasal medium with various substances, such as sonic hedgehog and retinoic acid, for five days. GNDF, BNDF, NT3 and other neurotrophic factors were added to further encourage motor neuron differentiation. After three to four days, rosette formations appeared in culture, a sign of neuroepithelial cell development. Cells derived from wild-type ESCs exhibiting neuroepithelial properties were later isolated to proliferate in media containing epidermal growth factor and fibroblast growth factor-2. Multiple NSC marker proteins were uniformly expressed by this group.

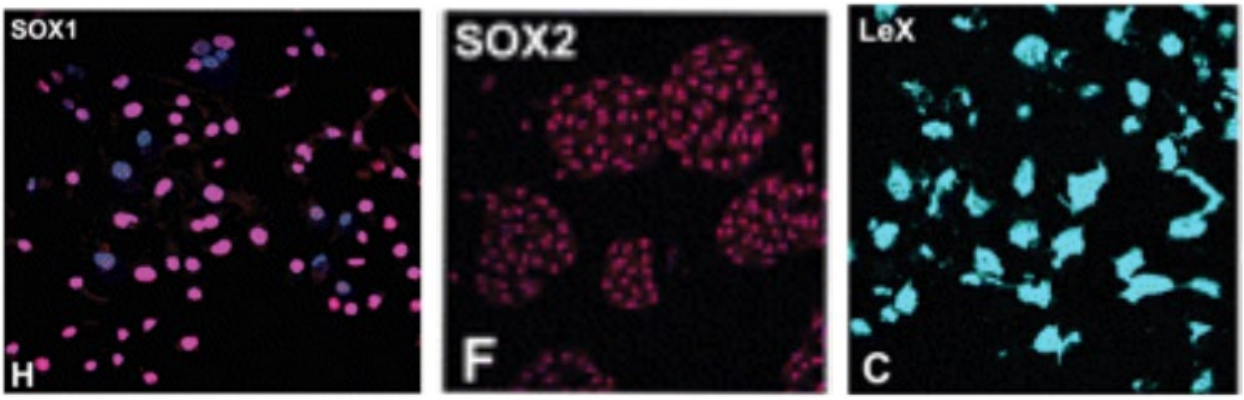

Figure 6. NSC Marker Protein Expression in Culture**.** Source: Corti et al., 2010**.** Description: NSCs derived from wild-type SCS were found to uniformly express various neural stem cell markers including Sox1 (H), Sox2 (F), and Lewis X (C).

OSG-embryonic stem cells went under additional treatment, where their genetic modification allowed ganciclovir and G418 to be used for purification of the culture into neuroepithelial cells. 88.5 ± 6.3% of the OSG-derived cells expressed Sox1 after four days, a significant increase from the 67.6 ± 5.6% of wild-type ESCs that expressed Sox1 after their seven day culture. NSCs from both groups were also transformed into motor neurons using a differentiation protocol involving neurotrophic and regional patterning factors. 27.9 ± 4.7%% of ESC-derived cells and 37.5 ± 3.5% of OSG-ESC-derived cells were ChAT and HB9-positive (indicating a motor neuron phenotype), showing overall improved differentiation.

Mice with SMA were injected with a total of 20,000 NSCs derived from either wild-type or OSG ESCs at one day of age. The increase in survival time of mice was significant, being 52.44% and 63.48% for the wild-type and OSG group, respectively, which was greater than that observed when using spinal cord-derived NSCs. There were also improvements in uprighting and locomotor behaviors in comparison to the treatment group. Mice were killed for tissue analysis once their health was severely declining. Upon inspection, some NSCs had amassed into clumps surrounding the meninges, but others successfully migrated into the spinal cord. In particular, transplanted cells were the most concentrated into the anterior horns of the spinal cord. a promising indication of their ability to migrate to damaged tissue. The number of GFP positive cells estimated to be in the spinal cord parenchyma of mice transplanted with NSCs-derived from wild-type ESCs was 2578 ± 212, whereas that in the spinal cord of mice with NSCs-derived from OSG-ESCs was 2976 ± 215. Acquisition of a motor neuron phenotype was still rare, however, as 356 ± 27 and 399 ± 28 ChAT-GFP cells were present in the wild-type ESC and OSG-ESC group, respectively. Cells were able to protrude their axons into white matter, and a few managed to elongate their projections into the ventral roots. Within both groups, there was no sign of teratoma formation during the 12 weeks mice were observed after injection.

Motor neuron number, size, and morphology between treated groups and the untreated SMA group greatly differed. Mice that received treatment had significantly less motor neuron reduction at day 13 of age (20.82 ± 3.68% for wild-type ESC & 17.14 ± 2.91% for OSG-ESC) than normal SMA mice (38.9 ± 2.96%). The diameter of motor neurons was also larger in transplanted vs. vehicle SMA groups. Again, similar to the effect observed by spinal cord-derived NSCs, myofiber quantity and diameter, as well as muscle area, improved. This created more evidence for a neuroprotective effect due to stem cell treatment, since transplanted cells made up only a small percent of the total number of motor neurons.

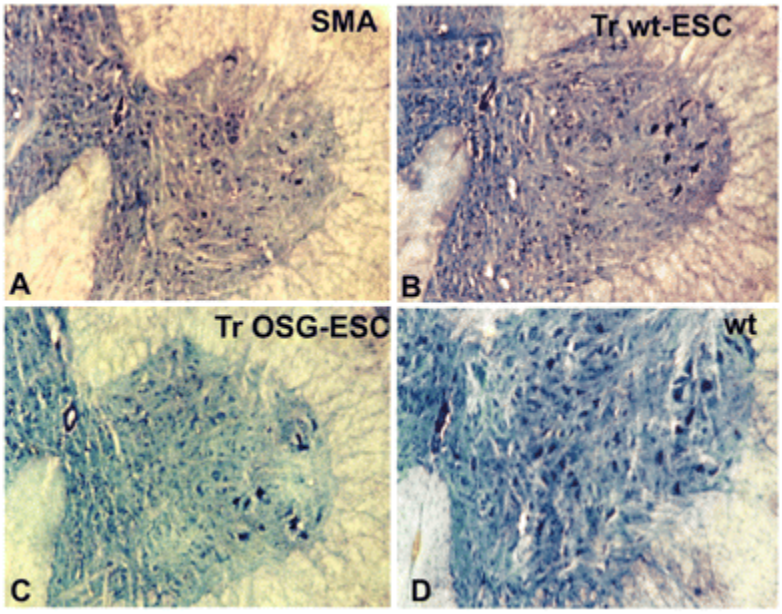

Figure 7. Lumbar spinal cord cross-sections from the different mice treatment groups**.** Source: Corti et al., 2010**.** Description: The amount and size of motor neurons within the ventral horn of SMA mice was increased through NSC transplantation from both OSG and wt-ESC sources. Progress was made towards the wt phenotype. (A) shows a ventral horn cross section of an SMA mouse, (B) shows that of an SMA mouse treated with wild-type ESC derived cells, (C) shows that of an SMA mouse treated with OSG-ESC derived cells, and (D) shows that of a wild-type mouse.

Indeed, the ESC-derived NSCs excreted much higher quantities of neurotrophic factors (GNDF, BNDF, and NT3) compared to astrocytes and fibroblasts in culture. Another co-culture experiment in which motor neurons from SMA mice were on the bottom layer and NSCs were on the top layer, with a porous membrane allowing factor diffusion between the two groups, revealed partial amelioration of defective axon growth that was observed in untreated SMA motor neurons. When neutralizing antibodies were used to disrupt the activity of GNDF, BNDF, TGF-a and NT3, both individually and together in the same co-culture system, the lengths of SMA motor neuron axons were reduced. When comparing NSC-treated groups to their vehicle and fibroblast counterparts, many of the aforementioned cytokines (GNDF, TGF-a, and NT3) had substantially boosted levels in the spinal cords of mice that received stem cell treatment.

This experiment reinforced the idea that neurotrophic factor expression could ameliorate SMA pathology while also showing improved motor neuron differentiation through PSC sources. Moreover, the use of these stem cells creates a much more accessible source in comparison to spinal cord-derived NSCs, though ethical considerations still need to be considered in translational application. Genetic modification of these ESCs produced better results in retrospect to the wild-type group and spinal cord-derived NSCs, indicating the superiority of drug-selected lines. OSG ESCs may have terminally differentiated more efficiently into motor neurons due to their genetic modification, while both groups could differentiate sufficiently into neuroepithelial cells. Data from the experiment suggests that the microenvironment of the SMA spinal cord promotes a motor neuron phenotype through internal signals, though it resulted in relatively limited motor neuron differentiation.

This stem cell treatment model still raises various considerations for how to increase amelioration from SMA pathology. While the therapy was done using 20,000 cells due to its safety and effectiveness, transplantation method advances could increase this number, potentially increasing the amount of cells engrafted into the spinal cord. Engraftment itself also creates another issue since the successful integration of NSC-derived cells within the spinal cord takes time, limiting the ability of neurotrophic protection to occur in early stages of disease. On the other hand, this

creates a better model for how effective disease amelioration could be when significant neurodegeneration has already occurred. Of course, research explicitly testing the difference in recovery based on date of transplantation would give concrete data on this matter, shedding light on the effectiveness of this NSC treatment if applied to newborn vs adult humans with SMA. Testing direct motor neuron transplantation instead of NSC treatment was an area of research as well, since it would no doubt allow substantially higher cell replacement if done correctly. There are various downsides, however, since motor neurons would have to be directly injected into spinal cord parenchyma instead of being less invasively injected into CSF like NSCs. This creates risks of additional harm, is more costly, and would be difficult to standardize as a treatment protocol (Kieran et al., 2004). NSCs have also been demonstrated to have extensive migratory abilities within the spinal cord once injected that aren't possessed by motor neurons(Corti et al., 2010). Though no comparison between these two transplantation sources was available in 2010, Corti et al. 's (2012) experiment testing motor neuron transplantation obtained from genetically corrected iPSCs has been able to characterize the effectiveness of the latter method.

Transplantation of Motor Neurons Derived from Genetically Corrected iPSCs

iPSCs from human SMA patients were genetically modified for increased SMN protein production, differentiated into motor neurons, and then transplanted into SMA mice models to determine if they could improve disease phenotype (Corti et al., 2012). Before this research, iPSCs created using viral vectors were shown to be capable of differentiating into motor neurons. However, insertional mutations caused by the vectors have the potential to disrupt regular cell activity. Additionally, tumorigenesis and altered differentiation may arise from transgene expression, making them unsuitable for clinical usage in a stem cell treatment. To overcome this issue, these researchers used non-viral vector methods to induce adult fibroblasts into iPSCs. Specifically, different plasmids that contained OCT4, SOX2, and other genes were placed into these cells, but were also lost over time, thus creating iPSCs without foreign DNA sequences. Then, single-stranded oligodeoxynucleotides were used to correct the differing nucleotides on exon 7 of the SMN2 gene. Two iPSC lines from SMA patients, a heterozygous line from the father of one patient, and a previously characterized wild-type iPSC line were used to generate motor neurons.

In the SMA iPSCs, about 4% of subclones showed 50% SMN correction, detected using observation of Gemini bodies (also known as gems, which require functional SMN protein to exist) and polymerase chain reaction (PCR). All iPSCs were differentiated into spinal motor neurons using the previously developed protocol for ESC cells.

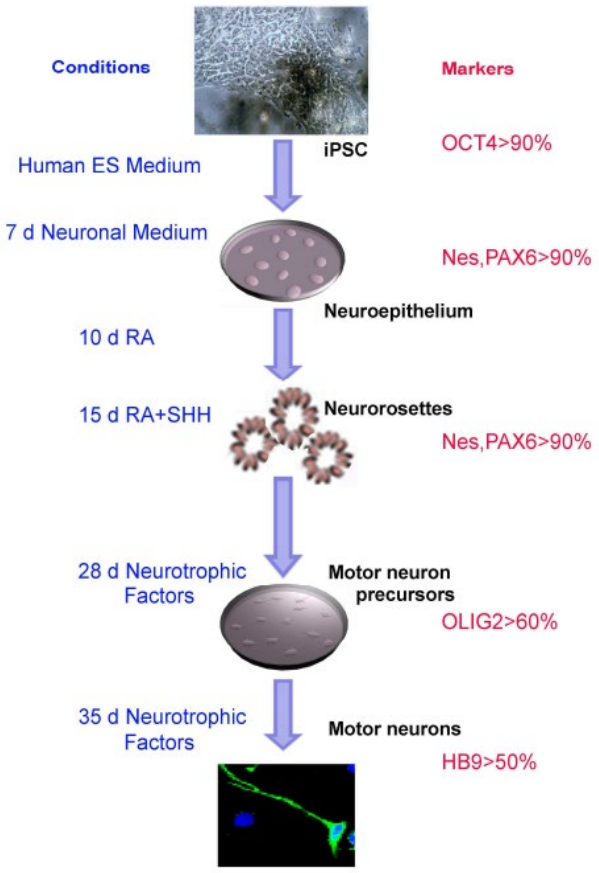

Figure 8. Differentiation Protocol for iPSCs Into Motor Neurons**.** Source: Corti et al., 2012**.** Description: Undifferentiated iPSCs expressing OCT4 amass into clusters that are then placed in neural medium for seven days. These primary NSCS form neurorosette structures and are also positive for PAX6. Next, cells are placed in a culture with retinoic acid and sonic hedgehog after first being dosed with retinoic acid. They developed into OLIG2-expressing motor neuron progenitors in week four, when they could be further differentiated by adding neurotrophic factors and reducing retinoic acid and sonic hedgehog. Most motor neurons expressed HB9.

A mixed cell population was achieved through this protocol, resulting in the purification of the culture using gradient centrifugation. Afterwards, with respect to ChAT and SMI32 expression (SMI32 is a motor neuron marker), there was no statistically significant difference between all four groups, with positivity being around 76%. Motor neuron cultures obtained from these different groups revealed defects in the untreated-SMA group (reduced axon growth and NMJ formation the latter observed when motor neurons were co cultured with myotubes) compared to wild-type and heterozygous groups, while also shown that genetically-corrected iPSCs experienced an amelioration of these defects. Further, gene expression analysis revealed that HET-iPSC and treated-SMA-iPSC motor neurons had little difference in expression profiles, whereas significant differences were found between the profiles of untreated-SMA-iPSC and HET-iPSC motor neurons. These pieces of evidence strongly suggest that oligonucleotide correction improves damage caused by nonfunctional SMN1 genes in motor neurons.

There were four groups, one control group of SMA mice that received a vehicle, and the other three being mice transplanted with either HET-iPSC, untreated-SMA-iPSC, or treated-SMA-iPSC derived motor neurons, all

receiving direct injection at one day old. Again, 20,000 cells were injected, and they were also modified to express GFP for tracking. The groups of mice that received transplantation were able to perform the grip test at day 13, which the vehicle mice could not do. Median life span for SMA mice were as follows: 21 for the HET group, 21, for the treated-SMA group, 19, for the untreated-SMA group, and 14 for the control group. These results indicate overall disease amelioration.

GFP motor neurons were detectable in the anterior gray matter of all mice spinal cords, and notably, there was an increase in engraftment comparing the treated SMA-iPSC group (approximately 3800 motor neurons) to the untreated group (approximately 2700). In the HET-iPSC group, donor neurons made up 27.5% of overall motor neurons while host neuron numbers also increased by 6.8%. Motor neuron size also increased, with the same effect being observed in the untreated and treated-SMA-iPSC groups. As the level of engraftment correlated to the neuroprotective effect, however, HET and treated-SMA-iPSCs gave greater enhancement than untreated-SMA-iPSCs. A small number of NMJs were also formed per spinal cord.

All three groups of iPSC-derived motor neurons could produce high amounts of NT3, VEGF, and NGF in comparison to fibroblasts, though they also excreted low amounts of the other neurotrophins (BDNF and GNDF). Another coculture tested the effect of secretory factors from the human motor neurons on primary SMA mouse motor neurons. Axon length and growth cone size were both increased by the treatment. SMA mouse motor neurons were then placed in microglial-conditioned media with and without the additional presence of human motor neurons. In comparing the two groups, it could be determined that human motor neurons supported SMA mouse motor neuron survival and axon growth through the expression of neurotrophins, even in a neurotoxic environment. Indeed, when the neurotrophin genes for NT3, VEGF, and NGF were silenced in motor neurons and transplanted into SMA mice, their therapeutic effect was reduced.

This transplantation model showed significant improvement in terms of increased motor neuron engraftment compared to previous experiments. Whereas ESC-derived NSC only made up about 7% of total donor neurons, the percentage for all iPSC groups was significantly higher (Corti et al., 2010; Corti et al., 2012). The creation of genetically corrected iPSCs is also critical for an enhanced therapeutic effect, since uncorrected SMA-iPSC derived motor neurons did not show the same capability for axon growth and exhibited decreased size. It is also important to note that though HET-iPSC derived motor neurons displayed slightly better results than corrected iPSCs, they are not clinically applicable for human treatment. Having a fully matched donor for cell transplantation is extremely important, as it minimizes the risk of graft-versus-host disease, in which the immune system attacks cells it views as foreign (Thiebaud, 2024). Also, no NMJs were formed in NSC transplantations, with there only being limited axon growth into ventral roots (Corti et al., 2008; Corti et al., 2010). On the other hand, direct motor neuron transplantation was shown to successful innervation of the skeletal muscles (Corti et al., 2012). While motor neurons were shown to exert a neurotrophic effect similar to NSCs, efficient connection of NMJs to their appropriate muscles is needed for this type of therapy to reach maximal effect. For proper motor neuron function, further research and experimentation testing cellular and external approaches to increase axon growth is a must.

Conclusion

The prospect of effective stem cell/stem cell-based therapy for SMA remains in the future. Though PSC sources were shown to be able to effectively differentiate into NSCs, and further into motor neurons in vivo, overall engraftment success has been limited. Even when directly injecting a total of 20,000 motor neurons, the highest recorded engraftment number within an SMA mice model was 4000 from HET-iPSC derived motor neurons (Corti et al., 2012). Migrating NSCs face the same problem, limiting the full neuroprotective potential of both types of therapies. Fixing this issue could require cellular modifications to promote increased homing to degenerated tissue or enhanced transplantation methods that allow the safe injection of larger cell quantities. The decision between transplanting motor neurons versus NSCs holds a debate in itself, with both having advantages in clinical application. The latter is currently unable to produce a large amount of motor neurons in the endogenous host environment, hindering significant motor function

restoration. Motor neuron transplantation is more effective in this regard, but may hold larger safety risks from intraparenchymal injection.

The spinal cord microenvironment in SMA is most definitely neurotoxic, creating potential issues in exogenous cell survival, but the use of current SMA treatments may be able to correct SMN production in glial cells, resulting in an improved milieu for engraftment (and increased motor neuron differentiation in NSC transplantation). To the study's knowledge, the exact effects that intrinsic signals within the spinal cord have on these cells have not been explicitly explained, and neither has there been an SMA animal model combining endogenous SMN protein correction with stem cell therapy, both of which offer further areas of research to improve the restorative effects of stem-cell therapy. Once engrafted, motor neurons then must effectively innervate skeletal muscles, an obstacle that needs to be overcome to create significant gains in motor function. Host environment modification through molecules, such as rolipram, have the potential to be combined with other modulations, including protein overexpression and exposure to axon-growth-enhancing substances.

Even with the significant improvement in SMA treatment and detection in recent years, stem cell therapy still proves to be a critical area of research. While early intervention is available, and can result in complete amelioration of SMA pathology, the high costs of Spinraza, Evrysidi, and Zolgensma make treatment difficult to receive, especially in countries without substantial healthcare programs. The expenses of these therapies mean that many people have gone on to undergo devastating neurodegeneration without a way to significantly regain their motor function. An effective stem cell transplantation could reverse their phenotype more than any current treatment. Furthermore, this therapy could be applied to other neurodegenerative diseases, such as ALS. The basic principles of inducing efficient neuron migration and axon elongation would no doubt be substantial in any stem cell treatment for neurological disorders in both the brain and spinal cord, not just those resulting in motor neuron deterioration. Though much more research is needed to make this treatment clinically viable, stem cell therapy offers a great reward for those who would benefit from it.

Limitations

The goal of this research is to assess the current development in stem cell transplantation therapy for SMA. The paper first explains the structure of the spinal cord and details the location of neurons and their processes. Next, stem cell basics, mainly concerning the three main types and their limitations, are discussed. The amelioratory effect of neural stem cells in spinal cord transplantation is then explained in the next section, along with notes about their characterization and reasoning for further differentiation into motor neurons for treatment. Afterward, the research details the spinal cord microenvironment in SMA and illustrates it as a potential issue in engraftment. However, it goes on to hypothesize a solution by combining transplantation with current SMA treatments. The challenge of axon growth once neurons have successfully engrafted into the spinal cord, as well as developments in overcoming this issue, are described next. Finally, different stem cell treatment mice models for SMA are summarized.

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