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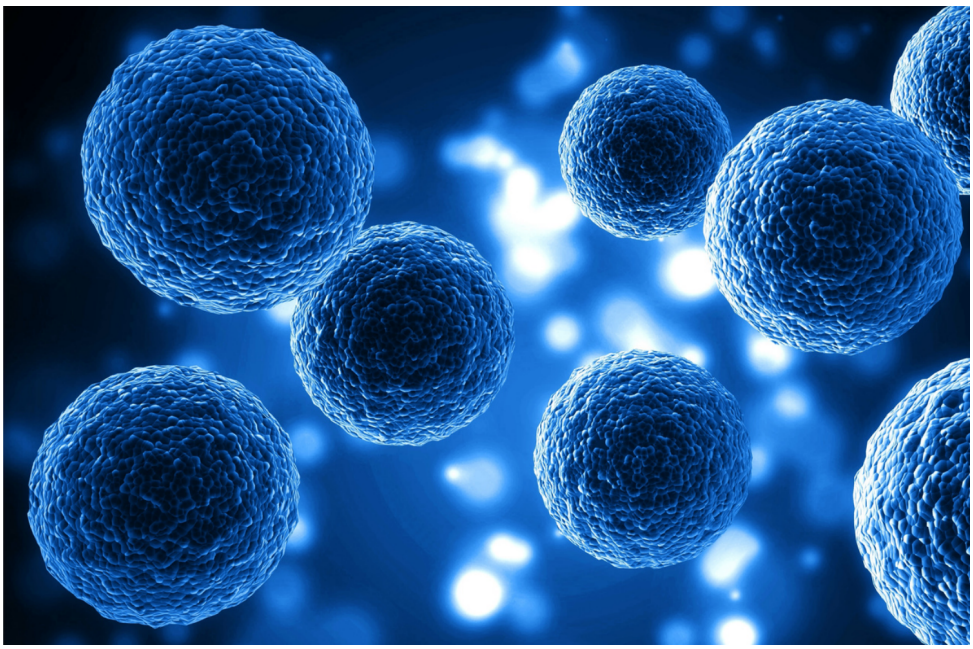
Administration of Mesenchymal Stem Cells as a Therapeutic for Amyotrophic Lateral Sclerosis

By: Tanvi Yalamanchili, Saahil Mohta and Donna Tran



Correspondence:
tanviy@berkeley.edu

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease caused by the degeneration of motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis, and eventually death. Exactly how and why motor neurons degenerate is still not clearly known, but certain pathogenetic mechanisms could explain the process. Current treatments and interventions, including pharmacological approaches, are not highly targeted and only offer short-lived benefits. No cure for the disease exists to date. However, mesenchymal stem cells, which are adult stem cells that are highly accessible and can differentiate into different types of tissue, are currently being studied. This review offers insight into some of the pathogenetic mechanisms, potential treatments, and how mesenchymal stem cell treatment could be the potential cure for ALS.

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1. Introduction

ALS is a progressive neurodegenerative disease caused by the loss of brain connectivity to the muscles. As motor neurons degenerate or die, the muscles weaken, eventually leading to paralysis[1]. Motor neurons are specialized brain cells located in the brain and spinal cord and control the motor function of the body. There are two types of motor neurons: upper motor neurons, which originate in the brain, and lower motor neurons, which send signals from the brain to the spinal cord and then to the target muscles[2]. The disease leads to the degeneration of upper motor neurons in the primary cortex and the degeneration of lower motor neurons in the brainstem and the spinal cord[1].

The word "amyotrophic" comes from Greek roots that mean "without nourishment to muscles" and refers to the loss of signals nerve cells normally send to muscle cells. Therefore, an important effect of ALS is the reduction of the blood-brain and blood-spinal cord barriers. These barriers are responsible for brain homeostasis, regulation of influx/efflux transport, and protection from damage caused by the debris in the bloodstream[7].

ALS was first discovered in 1869 by the French neurologist Jean-Martin Charcot but became more commonly known as Lou Gehrig's Disease after the famous baseball player Lou Gehrig was diagnosed with ALS at the age of 36. After being diagnosed, the Yankee player retired soon after as he experienced symptoms such as loss of strength, and loss of coordination, making him unfit on the field[3].

As a progressive disease, ALS causes motor neuron degeneration to worsen until the patient passes away[1]. Symptoms in the early stages of the disease include fatigue, poor balance, slurred speech, and tripping due to fasciculations (spontaneous muscle contractions/twitches) and atrophy (loss of muscle tissue)[4]. As some muscles paralyze, fasciculations continue, and joints start to become rigid and painful. Walking, eating and breathing start to become difficult. Some people have uncontrolled crying or laughter. In the late stages of the disease, the patients' voluntary muscles become paralyzed, and the muscles that help expand and contract the lungs for air are compromised, leading to respiratory failure[5,6].

Around 5000 people are diagnosed with ALS every year, and someone dies from the disease every 90 minutes. Once diagnosed, the average life expectancy is two to five years. Although it is most common for the disease to develop at ages greater than 60 years, it can occur at younger ages as well[6].

There are two types of ALS cases: familial ALS (fALS) and sporadic ALS (sALS)[7]. Familial ALS occurs due to genetic inheritance, and accounts for only 5-10% of ALS cases. It has an earlier age of onset compared to sALS[6,7]. It is known to primarily take form as a result of gene mutations such as SOD1 mutations. Over 20 gene mutations have now been identified that play a role in ALS disease progression. Silence Superoxide Dismutase 1 (SOD1) is a major gene correlated with ALS. Sporadic ALS on the other hand, although phenotypically indistinguishable from fALS, is not genetically inherited. As the most common form of ALS, sALS accounts for 90-95% of all cases[6,7].

Although the cause for ALS is unknown, the growing evidence points to the possibility of disease occurrence in humans due to gene-environment interactions. Exposure to certain toxic substances, viruses, or physical trauma could cause the disease[6]. Certain genes, known as "susceptibility genes," can trigger a neurodegeneration cascade upon interaction with certain environmental stimuli and/or factors. Unfortunately, no definitive environmental risk factors have been identified for ALS that can be replicated[8]. However, possible associations, based on clinical studies, have been identified between autoimmune pathology, neuroinflammation, head injury/trauma, metabolic disease, and ALS—specifically, the pathogenesis of ALS[8].

There are four main pathogenetic mechanisms that may explain the degeneration of motor neurons; these include mitochondrial dysfunction, glutamate excitotoxicity, oxidative stress, and inflammation. Current treatments include FDA-approved drugs such as riluzole and edaravone[9]. In addition, many treatments are being tested to reduce the effect of these mechanisms, including but not limited to, SOD1 gene therapy, and astrocyte transplantation[9]. However, the treatments mentioned above are

either short-lived or only target one of the pathogenetic mechanisms. Of the treatments being studied, Mesenchymal Stem Cells (MSCs) stand out as a potential cure for ALS. MSCs not only target the pathogenetic mechanisms to replace dead cells with healthy, differentiated cells but they also repair damaged cells[10].

Stem cells are unique in their ability to migrate to damaged tissues and/or sites, stimulate tissue repair and regeneration, and differentiate in response to extracellular signals. MSCs are adult stem cells that contain properties of self-renewal, immunosuppressive potential, and potency for trans-differentiation into motor neurons[10]. In particular, MSCs have been shown to be a promising therapeutic for ALS in preclinical and clinical trials. MSCs have the ability to stimulate tissue repair by differentiating into motor neuron cells, repair damaged neurons, and rebuild the brain-to-muscle connectivity[10]. This review offers insight into the different pathogenetic mechanisms of ALS, current treatment options, and why MSCs might offer a cure.

2. The Pathogenetic Mechanisms of ALS

2.1. Mitochondrial Dysfunction

There are a few pathogenetic mechanisms that may explain the disease progression. Of those, mitochondrial damage is an important pathogenetic mechanism to consider. Mitochondria is the major organelle responsible for many cellular processes such as producing energy, conducting cellular respiration, and maintaining calcium homeostasis[8]. This organelle is also known to play a key role in the process of apoptosis—a type of cell death activated by a series of molecular steps known as the caspase cascade. This pathway is often used by the body to remove unneeded or abnormal cells[11]. Because the mitochondria play a key role in regulating apoptosis, damage to this organelle within motor neurons can alter its ability to regulate the process. In ALS patients, the mitochondria that are present in their spinal motor neurons, skeletal muscles, and intramuscular nerves are swollen and vacuolated. Specifically, these swollen mitochondria activate the apoptotic caspase cascade, causing the death of the motor neurons[12].

Additionally, deposits of the misfolded SOD1 enzymes and altered protein expression in the mitochondria further alter the physiological function of the organelle[9]. A deficit in ATP production affects the cell's energy homeostasis and causes a defective energy metabolism. Alterations in the energy metabolism weaken the cell's health. Mitochondria with an altered structure or biochemical imbalance are prone to triggering apoptosis, thus leading to neuronal degeneration.

Mitochondria are responsible for regulating calcium levels as well. In ALS models, scientists have reported dysregulation of intracellular Ca^{2+} in motor neurons. Chronic elevations of cytosolic Ca^{2+} due to neuronal hyperexcitability elicit an increase in mitochondrial Ca2+ uptake and a decrease in mitochondrial Ca^{2+} efflux. When this condition is combined with altered mitochondrial calcium regulation, excitatory mitochondrial toxicity occurs, leading to dendritic mitophagy, mitochondrial depletion, and eventually dendritic atrophy and neurodegeneration[13]. Overall, Ca^{2+} dysregulation in motor neurons is at the cornerstone of disease progression of ALS[13].

2.2. Glutamate Excitotoxicity

Glutamate excitotoxicity is another pathogenetic mechanism that can provide an explanation as to how motor neurons degenerate in ALS patients. Glutamate is a principal excitatory neurotransmitter in the central nervous system (CNS) and plays a key role in triggering action potentials[14]. Neurotransmitters are chemical messengers that send signals from one nerve cell to a target cell, which could be a nerve cell, a motor cell, or a gland[15]. Glutamate sends signals within the CNS, which is made up of the brain and spinal cord. This neurotransmitter is originally generated in the presynaptic cleft terminal, exits through exocytosis, then moves across the synaptic cleft in order to activate postsynaptic receptors in the dendrites of postsynaptic motor neurons, which triggers action potentials[16,17]. The synaptic cleft is the space neurotransmitters travel through to send messages from one neuron to the next.

Glutamate reuptake transporters remove glutamate from the synaptic cleft to regulate the concentration of the neurotransmitter in the synaptic cleft,

getting rid of excitatory stimuli along with it[16]. High glutamate concentration in the synaptic cleft leads to excitotoxicity, which can either result from increased synaptic levels of glutamate or greater glutamate sensitivity in the postsynaptic terminal[9]. Overall, excitotoxicity occurs due to prolonged activation of glutamate receptors, which eventually leads to the degeneration and death of the motor neurons involved, induced through excessive neuronal firing[18]. ALS patients reported having lower levels of glutamate transporters, which leads to an increased concentration of this neurotransmitter in the synaptic cleft and an overactivation of glutamate receptors, determining the level of excitotoxicity within the neurons[7].

As a key regulator of neuronal activity due to the role it plays in apoptosis and neurotransmitter release, calcium plays an important role in excitotoxicity[19]. The activation of glutamate receptors opens calcium channels, allowing calcium to enter the cell. The calcium-buffering proteins in motor neurons make motor neurons sensitive to excitotoxicity. If intracellular calcium levels get too high, mitochondrial damage may result and lead to the activation of biochemical processes that affect neuronal degeneration[13]. Additionally, motor neurons are especially sensitive to excitotoxicity due to the high calcium permeability of these cells[13]. With more calcium able to enter the cells, intracellular calcium levels get high and lead to cell damage that induces the degeneration of motor neurons. Therefore, increasing the concentration of glutamate transporters would decrease the synaptic glutamate concentration and reduce excitotoxicity levels to help prevent degeneration[13].

2.3. Neuroinflammation and Glial Cells

Neuroinflammation is an immune response in the CNS to neuronal damage and plays an important role in the pathogenesis of ALS. An immune response is the body's way of fighting foreign substances to heal damage, but this can become harmful when accompanying a disease[20]. Neuroinflammation is characterized by overactivated microglia, reactive astrocytes, and infiltrating immune cells such as lymphocytes into the site of neuronal injury[9]. As the first line of defense within the CNS, microglia are activated and respond to signals released by damaged cells, more specifically

injured motor neurons[21]. Glial cells refer to the cells present in large quantities in the CNS and function as mediators of neuronal activity by surrounding and separating neurons; they can also play a part in regenerating damaged neurons[22]. Astrocytes are a type of glial cells that are closely associated with motor neurons. However, when the damage becomes worse, astrocytes and motor neurons release mutated SOD1 proteins, which activate pro-inflammatory microglia.

There are two kinds of microglia: M1 type microglia and M2 type microglia. M1 microglia are proinflammatory and secrete ROS and other neurotoxic molecules, which contribute to neuronal death[9]. By contrast, M2 microglia are anti-inflammatory and secrete neurotrophic factors—molecules that enable neurons to maintain connections with other neurons[23]. During the early stage of the disease, microglia with an M2 phenotype are present[24]. As the disease progresses, however, astrocytes and motor neurons activate microglia that present with an M1 phenotype[9]. These M1 microglia release ROS and other neurotoxic molecules that contribute to neuronal degeneration.

Additionally, astrocytes that are supposed to support neurons by using glutamate receptors to maintain low synaptic glutamate levels present differently in ALS patients who have astrocytes with the mutant SOD1 gene, which instead induces motor neuron degeneration. They downregulate the glutamate transporters, which in turn limit the amount of glutamate to be uptaken by the receptors out of the synaptic cleft[9]. They also secrete inflammatory molecules, and this inflammatory response further contributes to the alteration and degeneration of motor neurons[16]. While neuroinflammation is not the initial cause as to why motor neurons degenerate, it is a response to the damaged neurons that further exacerbates degeneration[20]. Reducing the inflammatory effects caused by overactivated microglia and reactive astrocytes could potentially be used to reduce neuroinflammation and slow neuronal degeneration.

2.4. SOD1 and Oxidative Stress

Oxidative stress is a cellular state motor neurons undergo that progresses and contributes to ALS pathogenesis. ALS patients have been shown to

have an increased level of oxidation, meaning they are in oxidative stress. Oxidative stress occurs when reactive oxygen species (ROS) accumulate within the cell or are being produced at a greater rate than they are removed, meaning the cell is unable to repair the stress[25]. This causes permanent damage to cell structures and macromolecules. The term ROS embodies superoxide, ozone, and all other reactive species that contain oxygen[26]. SOD1 is a major enzyme used to prevent oxidative damage[28].

Most neurodegenerative diseases are characterized by the cellular accumulation of misfolded proteins. In ALS mutant SOD1 proteins aggregate and alter normal cell function. The original function of the SOD1 enzymes is to reduce the reactive oxygen species (ROS) released from the mitochondria. They do this by catalyzing the conversion from superoxide into oxygen and hydrogen peroxide. This process plays an important role in the antioxidant defense of motor neurons[16]. However, in ALS, the mutant SOD1 enzymes no longer function as important molecules for antioxidant activity, but instead create oxidative stress. They convert antioxidants into superoxide by donating electrons from antioxidants to molecular oxygen. In this case, oxidative stress is not caused by a decrease or loss in the function of SOD1 but rather a new toxic function of the enzyme[9].

Not only is oxidative stress linked to the progression of ALS, it also contributes to the other pathogenetic mechanisms as well. When mutant SOD1 enzymes are present in the mitochondria, the mitochondria produces an abnormal amount of ROS, which, in turn, exacerbates oxidative stress[16]. Furthermore, this also determines excitatory damage and therefore motor neuron degeneration.

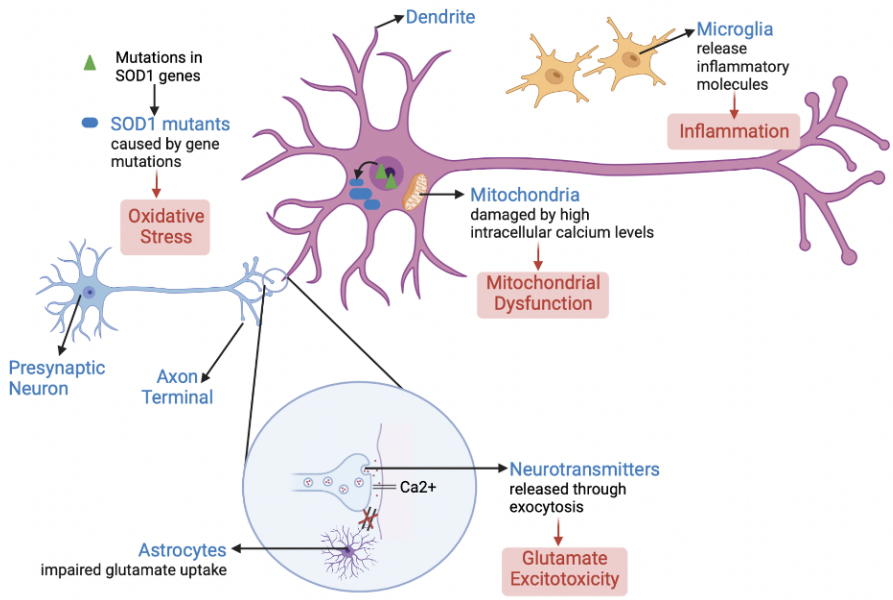

Figure 1. The four pathogenetic mechanisms of ALS: Mitochondrial Dysfunction, Glutamate Excitotoxicity, Inflammation, Oxidative Stress.

3. Novel Cell Therapies

3.1. Pharmacological Approaches

Pharmacological therapies for ALS target neurodegeneration by stagnating or impeding the death of motor neurons. Riluzole is the first FDA approved drug for ALS after it initially proved to be successful in clinical trials in the 1990s[28]. Common brand names of riluzole are Exservan, Rilutek, and Tiglutik. Since its successful administration, new drugs including edaravone and AMX0035 have been studied to evaluate their efficacy against predecessors.

Riluzole functions by protecting motor neuron cell lines from glutamate stress. Glutamate is an essential neural excitatory neurotransmitter which facilitates proper signal propagation throughout the central nervous system[29]. Imbalances in glutamate levels can affect nerve health and contribute to cell death. Like many neurodegenerative therapeutics, riluzole prevents excitotoxicity and slows degeneration of motor neurons. The most prominent clinical trials involving solely riluzole as a therapeutic for ALS are from the late 20th century where the drug was initially found to be a successful treatment. In a 1994 clinical trial, conducted before the FDA approval of riluzole in 1995, riluzole slowed ALS progression by inhibiting

disease processes in the CNS[30]. Factors including death rate and deterioration of muscle strength were higher for the placebo group than those treated with riluzole in a one year period. The study also noted that a subsequent experiment on dosage should be conducted to which researchers in 1996 tested the impact of riluzole dose-ranges on ALS patients to determine that the prescription amount with the best benefit-to-risk ratio is a 100 mg dose, further confirming the success of riluzole.

While a complete understanding of riluzole's mechanism of action has not been established, properties including its ability to inhibit glutamate release and inactivate voltage-gated sodium channels associated with damaged neurons are attributed to its effectiveness for ALS[31]. Neuroprotective drugs like riluzole function by binding to voltage-gated sodium (Na_v) channels to keep them in an open and inactivated conformation[32]. It also inhibits Na^+ currents which regulate excitability imbalances to prevent neuronal damage. A common brand of riluzole is RILUTEK® which offers the medication in the form of a prescription pill. In 1995, it became the first drug to receive FDA approval for a treatment of ALS as it proved to "extend survival and/or time to tracheostomy" in clinical trials[33]. In the original clinical trials, it was estimated that the patients treated with riluzole have longer survival times of two to three months. However, a retrospective statistical analysis on population studies of ALS compared the mean survival rate between treated and non-treated patients to suggest that riluzole may even extend survival by six to 19 months, longer than the original expectancy[34].

Although riluzole is a favorable option, advances in pharmacology have been geared towards new drugs and additive treatments to improve efficacy. In May of 2017, the FDA approved the second targeted drug intended to slow ALS progression: edaravone. The most common formulation is RADICAVA IV which has shown signs of selective effectuality[35]. Unlike riluzole which targets excitotoxicity, edaravone is an antioxidant– a compound inhibiting lipid oxidation to prevent the production of free radicals and chain reactions that may induce cell damage[36]. By reacting with radical species, this antioxidant captures unstable reactive oxygen species because a large concentration of these molecules contributes to the

degeneration of motor neurons. An active phase 3 clinical trial is currently evaluating the safety and efficacy of oral edaravone for ALS patients based on the ALS Functional Rating Scale- Revised (ALSFRS-R) score which observes lifestyle changes such as independence, speech, and mobility[37]. The first version of this drug was administered via intravenous infusion, a drug-delivery method administered directly into a vein using a cannula, which received FDA approval on May 5, 2017. The doses are given in slow and set treatment cycles to observe for signs of hypersensitivity. The infusion can take up to an hour and must be performed by a healthcare provider. On May 12, 2022, Radicava Oral Suspension was FDA approved, which grants the user more flexibility in administering treatment.

Prior to Radicava's oral formulation, differences between the two treatments such as absorption and distribution were more observable[38]. While riluzole is in a more convenient tablet form, injections of edaravone do not face issues with absorption, and there may be more transport across the brain blood barrier (BBB), a semipermeable membrane facilitating the transport of molecules and substances from the bloodstream to the brain. Cohort studies have also observed the effects of using intravenous edaravone in conjunction with riluzole, but there was no improvement in ALS in comparison to standard riluzole therapy[39]. Since the issue of convenience is no longer a dividing factor, new research is geared towards testing the variation between both oral forms in efficacy for ALS treatment.

Another novel drug that is currently being tested in clinical trials is AMX0035, used as an oral combination therapy of sodium phenylbutyrate (SPB) and taurursodiol (TURSO) to block cell death mechanisms[37]. AMX0035 was developed by Amylyx Pharmaceuticals, Inc., and on September 29, 2022, the FDA approved this drug as the newest therapeutic for ALS[41]. It will be sold to patients in the U.S. under the name Relyvrio, and it proved successful in clinical trials, with patients reporting less ALS symptoms under the treatment in comparison to a placebo[40]. In the briefings between the company and the FDA, it was also noted in the results of their clinical trials that patients who were randomly assigned to receive AMX0035 also showed statistically significant increases in survival duration of around five months longer than the placebo. This value is comparable to

the survival benefits of riluzole and edaravone which are three and six months, respectively. After nearly a decade of developing a new combination drug for ALS, Amylyx's co-founders Justin Klee and Josh Cohen became successful at providing the ALS community with newfound hope for an improved lifestyle.

This drug targets two of the various mechanisms contributing to disease propagation: ER stress and mitochondrial stress. SPB alleviates toxicity from ER stress, increasing the number of heat-shock proteins (HSPs) which provide neuroprotection[42]. Likewise, TURSO reduces mitochondrial stress by increasing the mitochondrial selectivity and cell's threshold for apoptosis. In a clinical trial conducted by the Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS) and Amylyx Pharmaceuticals, patients received a 2:1 ratio of SPB to TURSO dissolved and taken orally or through a feeding tube. After the six month period of daily medication, there was a reduction in the rate of dysfunction based on the ALSFRS-R. ALS patients involved in this trial were still taking riluzole, edaravone, or both, but researchers did not observe notable differences in efficacy. In the near future, more information on AMX0035 for ALS treatment may be obtained if it receives FDA approval and becomes accessible to ALS patients as a viable option alongside riluzole and edaravone.

Each ALS therapy has its own benefits and caveats, and due to the diversity in ALS types and ALS patients, one form may prove to be more effective in reducing neurodegeneration than other. As the first drug for ALS, riluzole remains more effective with extensive results from clinical trials to prove its efficacy. Advancements in the development of newer drugs such as edaravone and AMX0035 provide increased confidence in the possibility of slowing the rate of ALS progression. Similarly, stakeholders are increasingly interested in new therapies for increased access and improvements on the ALS rating scale. While none of the drugs approved for ALS are a complete cure because they solely function to delay disease propagation, new approaches are still being tested for factors including an increase in survival probability, safety, and long-term consequences, riluzole still remains the most prevalent treatment.

Pharamcological Therapeutics

Medication	Brand Name(s)	Function	Availability	Main Side Effects	Efficacy
Riluzole	Rilutek (1995) Tiglutik (2018) Exservan (2019)	Inhibits glutamate release Binds to and inactivates Na_v channels	Oral tablet Oral suspension Oral film	Numbness Loss of strength Nausea Drowsiness	Prolongs survival in early-stage ALS
Edaravone	Radicava (2017) Radicava ORS (2022)	Inhibits lipid oxidation	Intravenous injection Oral suspension	Hypersensitivity Contusion Gait disturbance	Slows progression of ALS symptoms
AMX0035 *awaiting FDA approval	Albrioza (*2022)	Alleviates ER and mitochondrial stress	*Dissolved oral suspension	Nausea Diarrhea Abdominal pain	---

Table 1. A comparison table of the drugs riluzole, edaravone, and AMX0035 for ALS

4. Gene Therapy

Since pharmacological therapeutics have yet to eradicate and cure ALS, gene therapy approaches have also been explored as a potential solution. Gene therapy can be divided into two types: (1) non-viral gene therapy where nucleic acid sequences are delivered to the host cell for pathological modification; and (2) viral gene therapy in which genetically-modified viruses are used as viral vectors to deliver sequences. Antisense oligonucleotide (ASO) drugs belong to category one and are used in pre-clinical trials of *SOD1*-fALS. Superoxide dismutase 1 (*SOD1*) is the first of 30 ALS-related genes currently discovered. Oxidative damage is a result of an abundance of ROS such as superoxide in the cell, making it unable to prevent cellular stress. *SOD1* prevents oxidative damage and reduces superoxide leakage from mitochondria by catalyzing the conversion of superoxide into oxygen and hydrogen peroxide. Mutations in the *SOD1* gene contribute to the pathogenesis of familial ALS and accounts for 15–20% of fALS cases, and begin with the misfolding of the gene which causes it to be degraded by the ubiquitin/proteasome system (UPS)[18]. However, once a large concentration of misfolded proteins accumulate, this disrupts proteostasis–the regulation of proteins from synthesis to degradation–and retriggers autophagocytosis to increase the number of autophagosomes for the removal of dysfunctional cellular constituents[16]. Over time, an accumulation of mutated *SOD1* proteins induces cell stress responses, resulting in disease pathogenesis. A buildup of misfolded mutant SOD1 proteins in the mitochondria can adversely affect physiological function of the organelle. Specifically, studies have noted irregularities in the

production of ATP and ROS which signal cell survival and cell death, energy and calcium homeostasis, and apoptosis in mitochondrial transport along axons[9].

An ASO used to target *SOD1* mRNA is Tofersen (BIIB067), previously 'IONIS-SOD1Rx.' It relies on Ribonuclease H (RNase H), an endoribonuclease enzyme that cleaves the RNA from the RNA/DNA hybrid, to degrade *SOD1* mRNA and prevent the production of toxic SOD1 proteins[18]. In a phase 1/2 clinical trial, ALS patients with a *SOD1* mutation received multiple doses of Tofersen, and results showed that patients treated with the greatest concentration of the ASO had lower *SOD1* protein levels by 36%. Also, a decline in the reduction in the ALSFRS-R score was observed. However, since the drug was delivered via an intrathecal lumbar puncture, adverse effects including headache and back pain were common consequences. In another approach, researchers relied on a lentivirus encoding for an RNA silencing (siRNA) gene that catalyzes the selective degradation of *SOD1*-mRNA. The injection of this virus in the muscle or directly in the spinal cord of transgenic mouse models of ALS showed reduced *SOD1* expression delaying the neurodegeneration, but data about the slowing down of the disease progression and survival is controversial and requires further study[9].

Gene therapy targeting the many ALS-related genes can prevent disease proliferation by directly preventing mutations at the source. *SOD1* mutations can lead to excitotoxicity, mitochondrial dysfunction, and ER stress which increases the likeliness of motor neuron degeneration. The appeal of this form of treatment is that the different genes can be individually suppressed by ASO's like Tofersen to reduce the degree of toxic variants. To optimize gene therapy for ALS, the pathological mechanisms and roles that the genes play must be well understood and develop a barrier for further propagation of neurotoxic factors. The limitations and challenges associated with this approach are that it is mostly used to treat fALS cases and very few sALS cases.

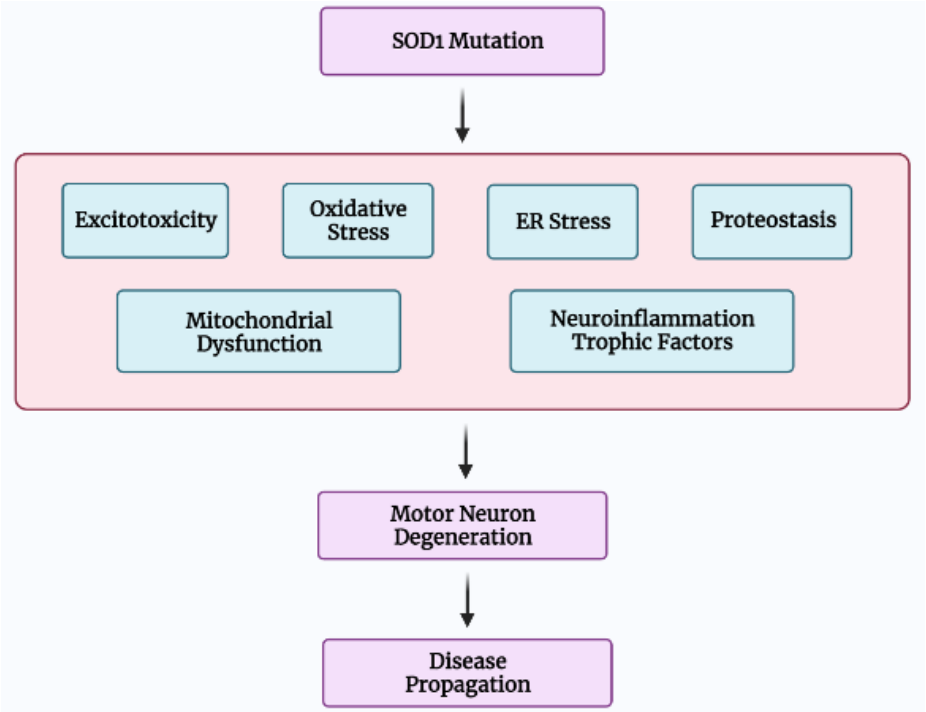

Figure 2. Pathways linking SOD1 mutations to ALS pathology

5. Astrocyte Transplantation

The most abundant glial cells within the CNS are astrocytes, a form of brain cell that regulates neural functions through signal diffusion. Correspondingly, they are necessary in the CNS for neuronal survival. Any changes including stress or injury to the CNS cause astrocytes to transition from healthy astrocytes to A1-type neurotoxic astrocytes which promote degeneration of motor neurons[43]. The progression in toxicity of healthy motor neurons causes a loss in homeostatic functions or gain of toxic functions. Astrocytes of ALS patients are characterized as A1-type, and targeted therapies to control the proliferation of neurotoxic astrocytes are currently being tested for ALS.

Astrocyte-targeted therapy involves restoring the function of neurotoxic astrocytes through the transplantation of healthy astrocytes[43]. While this decreases the concentration of misfolded proteins and aids in neuroprotection, there is also the risk that these healthy astrocytes can undergo the transition to A1-type astrocytes when they are introduced to the CNS of ALS patients. This new environment can be toxic to the new cells due to the large accumulation of misfolded proteins. Although it has

been deduced that astrocytes secrete neuroprotective factors that diffuse to the motor neurons, later mechanisms including their attachment and long-term survival need to be further explored.

In a phase 1/2A trial, researchers from Cedars-Sinai Medical Center are hoping to find a long-lasting solution to ALS by testing the effectiveness of their engineered neural progenitor cells which differentiate into astrocytes inside the body to secrete the GDNF protein, promoting astrocyte survival[44]. CNS10-NPC-GDNF are progenitor cells which will release paracrine factors inside the body. As they release GDNF, this neuroprotective factor is also transferred to dying motor neurons in the motor cortex. Two doses of adult stem cells will be delivered to the cortical area of the human primary motor cortex (M1) as researchers observe for signs of repaired upper motor neurons and improvement in hand function. Signs of potential efficacy will be determined by comparing the difference in rate of loss of hand strength between the two sides. Studies relating ALS-genes to astrocyte therapies noted that mSOD1-expressing astrocytes induced selective death of spinal motor neurons, so a potential solution could be the transplantation of healthy astrocytes or selective silencing of the mSOD1 gene in astrocytes.

While many hypotheses are developed, it is believed that transplanting healthy astrocytes will generate a better environment for the motor neurons. Therefore, the molecular pathology of astrocytes in ALS patients needs to be further studied. Once the healthy cells are delivered, the pathway it takes for diffusion and specific function remains unknown[45]. While this treatment is appealing because it has the potential of restricting the propagation of toxic cells and slowing degeneration, transplanted cells can still transform into A1 reactive astrocytes once they are introduced to a more neurotoxic environment. Under excitotoxic conditions, ALS neurons and astrocytes can be more sensitive to neuromodulation, so it is pivotal to developing novel cell therapies for ALS.

6. Stem Cells

Because the focus of this paper is the application of mesenchymal stem cells (MSC) as a therapeutic for ALS, it is imperative to broadly consider stem

cells, their capabilities, and their advances in pertinence to regenerative medicine (a process by which healthy cells are introduced in order to replace cells that have been adversely impacted by disease) and disease therapeutics[46]. Stem cells are cells from which differentiated daughter cells, or cells with a specialized function, can be engendered within the human body. Daughter cells are cells that result from the mitotic or meiotic reproductive division of a singular cell, and possess a specific function; examples include epithelial cells, adipose stromal cells, and smooth muscle cells. Furthermore, these cells are undifferentiated and are typically sourced from adult, fetal, and embryonic tissue, in addition to differentiated somatic cells[47].

There are four main forms of stem cells: Adult, Embryonic, Induced Pluripotent, and Mesenchymal[48].

6.1. Adult Stem Cells

Adult stem cells are commonly regarded as tissue-specific or somatic stem cells, and are found in a wide range of tissues, including the brain, heart, liver, and bone marrow. They can facilitate the creation of various cell types upon injury, but the degree to which this differentiation can occur is limited in comparison to embryonic stem cells. Adult stem cells are multipotent, or limited to differentiating into the specialized cell type in the tissue or organ of residence. They can also be utilized for general maintenance of tissue and organs and have the capacity to maintain and repair organs and bodily tissue, which can give rise to cell regeneration, thus indicative of the role stem cells can play within regenerative medicine[49, 50].

6.2. Embryonic Stem Cells

Embryonic stem cells (ESCs) are conventionally sourced from embryos three to five years of age and are more versatile in comparison to adult stem cells because they are pluripotent, or capable of giving rise to a variety of cell types not limited to their organ or tissue of residence. As a result, they are more versatile and have the capability of tissue/organ regeneration and repair[51]. However, embryonic stem cells have a higher probability of being rejected by the host, and their use is ethically controversial by virtue of the

fact that the destruction of human embryos is necessary in order to obtain ESCs[52].

6.3. Induced Pluripotent Stem Cells

Induced pluripotent stem cells are a class of pluripotent cells that are commonly derived from blood or skin cells and have been genetically engineered into an embryonic-like state in order to behave as ESCs[53]. Mesenchymal stem cells, the subject of this paper, are a subgroup of adult multipotent stromal cells that are derived from adipose tissue, umbilical cord tissue, amniotic fluid, and bone marrow, and have the capacity to differentiate into numerous cell lines[54]. Stromal cells comprise the connective tissues that surround bodily tissues and organs and are separate from the stroma.

Stem Cells

	Adult	Embryonic	Induced Pluripotent	Mesenchymal
Derived From	Bone marrow or fat	Embryos three to five years of age	Blood or skin cells (also genetically engineered into an embryonic-like state)	Bone marrow, skeletal tissue, placenta, adipose
Pluripotent vs. Multipotent	Multipotent	Pluripotent	Pluripotent	Multipotent
Advantages	Can facilitate the creation of various cell types upon injury	More versatile Capable of tissue/ organ regeneration and repair	Versatile like embryonic stem cells Non-controversial	High availability High differentiation plasticity Self-renewing Less susceptible to tumorigenesis
Disadvantages	Limited degree of differentiation	Higher probability rejection by the host Morally controversial	Observed higher rates of cell death when isolating IPS cell lines	High selectivity required for isolation High risk Irreversible

Table 2. A comparison of adult, embryonic, induced pluripotent, and mesenchymal stem cells

Stem cells are especially significant and have been the subject of intensive study due to the fact that they are the only cell that can naturally develop and differentiate into varying cell types primarily through the autocrine production of immunomodulatory molecules, bioactive molecules stored within extracellular vesicles, and growth factors such as the vascular endothelial growth factor (VEGF)[47]. As a result, stem cells could, theoretically, limitlessly participate in cell division in order to replace cells within damaged tissue[55]. They have the unique ability to differentiate in response to the microenvironment in which they have been directed

toward[56]. It is also important to consider, though, that stem cells need to be carefully selected according to their biological capacity to survive within the host organism (where the cells are transplanted), and their ability to differentiate and migrate to various tissues. The upsides and downsides of each form of stem cells must be evaluated upon consideration for disease treatment or regeneration. On an interdisciplinary scale, however, because the treatment options for neurological diseases remain heavily limited and drug approval rates remain poor in comparison to other therapeutic areas, the implementation of stem cells carries immense potential for patients afflicted with disorders such as ALS. Furthermore, numerous degenerative diseases involve an improper functioning or loss of specialized cells within the body and there is an imbalance between the supply and demand of potential donors and their cells within their organs or tissues. Specifically, the supply of donors is currently unable to fulfill the demand of replacement cells. As such, stem cells could serve as a solution, continuously producing viable cells that could replace damaged or injured tissues and thus address a number of afflictions[55].

To date, stem cells have been implemented for the treatment of diseases such as amyloidosis, germ cell tumors, and certain cancers through autologous (obtained from the same individual) and allogenic (obtained from a source outside the individual, such as a donor) transplantation. With cancers, specifically, stem cells have been implemented to replace cells that have been adversely impacted by the cancer pathogenesis itself or chemotherapy. While stem cells are continually being utilized for the treatment and maintenance of a myriad of diseases, further research is necessary to fully elucidate the pathogenetic mechanisms by which stem cells function, to properly isolate and identify stem cells to avoid immunological rejection to the greatest extent possible, and to address the predisposition of induced pluripotent stem cells to tumorigenesis, or the formation of tumors in vivo[57, 55].

6.4. Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are a class of stem cells - multipotent and non-hematopoietic stromal cells - which are conventionally derived from adult and fetal tissue, such as skeletal muscle, the placenta, blood, adipose, and the umbilical cord[7]. MSCs have been demonstrated to take residence

within most connective tissues in the human body. This high availability within human tissues makes MSCs accessible as a disease therapeutic. Since MSCs are multipotent, they have a high differentiation plasticity, or a capability of trans-differentiating into multiple specialized cell types[58].

Transdifferentiation is the process by which a cell can be made to differentiate from one cell type into another through genetic reprogramming. For instance, they are able to differentiate into nerve, heart muscle, liver, and endothelial cells, in addition to the germ layers - the ectoderm, mesoderm, and endoderm. Furthermore, MSCs are capable of differentiating into neural, glial, and astrocyte-like cells, which can be significant in pertinence to their utilization as a treatment for ALS and similar neurodegenerative diseases as this indicates their ability to effectively function as motor or dopaminergic neurons. This in-vitro differentiation potential of MSCs to generate a myriad of cell types that could replace lost cells within injured tissue has been a significant justification for the use of MSCs themselves[59].

MSCs possess several advantages which increase their potential of being implemented in disease therapeutics, such as an ability of self-renewal (the process by which stem cells continually divide to produce additional undifferentiated stem cells), their ease of isolation, and a decreased susceptibility to tumorigenesis (formation and accumulation of malignancy and malignant properties within cells). In addition, there is no specific immunosuppressive treatment that is necessary or otherwise warranted upon the transplantation of MSCs, as demonstrated by the fact that studies have indicated that MSCs are immunosuppressive both in vivo and in vitro[9,60]. Typically, MSCs employ this immunosuppressive effect on T cells, which are a major component of the adaptive immune system. Most significantly, however, the therapeutic effects of MSCs are largely associated with their immunomodulatory effects, which include MSCs' regulation of lymphocytes that are associated with the innate and adaptive immune system[8].

Immunomodulation is the modulation or alteration of the immune system, as determined by the activation or suppression of immunomodulating

agents such as cytokines, vaccines, and monoclonal antibodies. Specifically, MSCs are able to regulate T cell proliferation and have the potency to upregulate regulatory T cell function, thus simultaneously highlighting their immunosuppressive abilities because the regulatory T cells function to suppress the body's immune response, allowing for a maintenance of homeostasis. For this reason, there have been proposals for the application of MSCs to the treatment of autoimmune diseases, which are a category of diseases in which healthy cells are attacked by the body's immune system. MSCs also regulate their immunomodulatory function in accordance with micro environmental and inflammatory conditions, indicative of the high level of therapeutic plasticity of MSCs (they have a potential of being applied in order to treat a myriad of medical conditions).

MSCs function upon transplantation through various methods. One such method is cell fusion, which is the process of a cell interacting with nearby cells in order to form a multicellular assemblage with a universal function. For instance, the fusion of MSCs with rodent Purkinje cells was observed, and these fused cells were implemented to improve the therapy of neurodegenerative disorders and disorders involving the cerebellum[56]. Furthermore, mitochondrial transfer is a unique mechanism of action of MSCs, and is the process in which cells transfer mitochondria to neighboring injured cells (thus replacing damaged mitochondria and aiding in the restoration of cellular function). Additionally, the secretion of extracellular vesicles (exosomes) by MSCs is a similarly important mechanism of action. MSC exosomes are secreted into the extracellular space with multivesicular bodies within MSCs fused with the cellular membrane[59]. Extracellular vesicles have the potency to transport essential macromolecules such as genetic material to neighboring cells through endocytosis to maintain physiological homeostasis, and as such, carry a potential therapeutic application. Studies have demonstrated that the exosomes secreted by MSCs possess anti-inflammatory and regenerative properties in pertinence to traumatic brain injury, stroke, perinatal brain injury, and wound healing[61], highlighting that MSC-derived exosomes are crucial to the proper function and therapeutic properties of MSCs as a whole.

There are certain characteristics of MSCs that aid in their applications within regenerative medicine[56]. MSCs have the ability to form colonies, which can be measured by the term "colony forming unit" (CFU). A CFU is a measure of the number of viable cells within a sample that are able to multiply through binary fission. This is one of the primary functional characteristics of MSCs, which highlights their ability to proliferate upon transplantation and which would reasonably eliminate (1) the need for consecutive transplantations and (2) possible supply shortages associated with the availability of MSCs. In addition, while it has been discussed above that MSCs have a high differentiation plasticity as compared to other stem cells, MSCs have the unique capability of undergoing trilineage differentiation into adipocytes (fat cells which store energy as fat), chondrocytes (key component of cartilage tissue), and osteocytes (bone cells which make up approximately 90-95% of cells within bone tissue). Furthermore, the telomerase activity of MSCs is significant as their activity is typically augmented within MSCs. Telomeres are the end caps of chromosomes which shorten with cell replication and division, and similarly, telomerase is the enzyme responsible for the region of the telomeric region at the 3' ends of chromosomes. Telomeres also protect chromosomal ends from degradation and improper DNA recombination (which can result in apoptosis). As such, an increased telomerase activity is directly correlated with a longer cell lifespan. Since the telomerase activity is augmented within MSCs, MSCs have a longer period of survival. The telomerase activity and the telomere lengths can also be analyzed as a quality control measure to aid in the selection of MSCS for therapeutic applications.

Studies have demonstrated that MSCs have the capability and potential of being trans-differentiated into neuron-like cells; however, the specific protocols for inducing this differentiation involve toxic chemicals and thus cannot directly be utilized within humans. As such, further research and study of the differentiability of MSCs is warranted when considering their potential as a viable therapeutic for ALS and more broadly, neuron degeneration.

7. Mesenchymal Stem Cell Treatment for ALS

Mesenchymal stem cells could be the potential cure for ALS not only due to its ability to stimulate tissue repair by differentiating into motor neurons but also by repairing damaged cells within the body. MSCs are especially advantageous compared to other stem cells due to many of its properties. One of these advantages include its great availability—they can be derived from adult tissue such as bone marrow tissue, skeletal tissue, or adipose (fat) tissue or can also be derived from fetal tissue such as the placenta or umbilical cord. Because of these specific tissue sources, MSCs can be found in abundance, especially due to the medical waste created from these tissues. For example, adipose tissue can be obtained from liposuction while the placenta is delivered and most of the time gotten rid of. However, bone marrow tissue—the soft, spongy tissue found inside the bone and contains stem cells[62]—can be obtained in the most abundance and is even possible to be extracted from the patient itself to mitigate the chance of rejection by the host.

Although MSCs are multipotent, they can exhibit pluripotent properties[63]. There is growing evidence that MSCs can transdifferentiate into motor neurons. One study shows that a cocktail of basic fibroblast growth factor (bFGF) and retinoic acid (RA) with human MSCs (hMSC) provide the most effective and efficient transdifferentiation of MSC into neural cells[64]. Two days after injection of these cells, these cells expressed glial markers, and 12 days after injection, 90% of the hMSCs differentiated into cells that expressed neuronal markers, which also include transcription factors linked to aid the development of the differentiated neurons[65]. This confirmed the ability of MSCs to be able to differentiate into neurons, providing a method to stimulate tissue repair.

After being injected intrathecally, in order for the MSCs to differentiate and develop in the correct area, ALS allows for an effective stimulus. A key advantage of MSCs over similar forms of stem cells is their ability to migrate towards inflammatory foci through the expression of chemokine receptors, and as a pathogenetic mechanism of ALS, inflammation provides MSCs the knowledge of the damaged site[66]. Chemokines are a family of cytokines which stimulate white blood cell migration to sites of infection and thus

play an integral role in the homeostasis of the immune system[67]. Chemokine receptors interact with the chemokines, themselves, in order to manage the function of the immune system; it is thus evident that the expression of chemokine receptors by MSCs plays a key role in the reduction of inflammation to maintain equilibrium within the immune system.

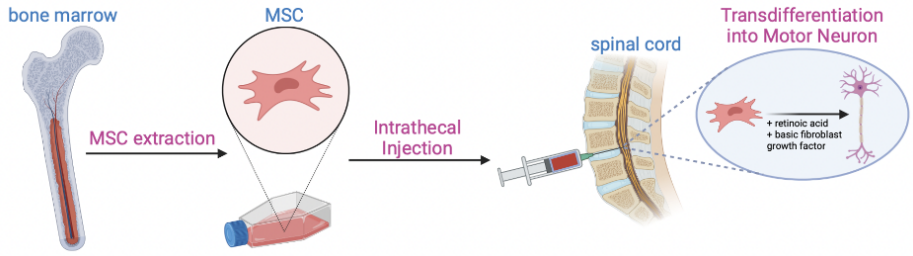

Figure 3. Mesenchymal stem cells are extracted from bone marrow and are intrathecally injected into the lateral spinal cord, where they can transdifferentiate into motor neurons.

MSCs not only stimulate tissue repair by differentiating into motor neuron cells but can also repair damaged cells and prevent motor neuron degeneration from taking place to cure the disease. Human and animal MSCs showed promising results in regard to using these stem cells for neural repair and not just to replace the damaged motor neurons. One way MSCs do this is by producing trophic factors, which can repair or regenerate tissue[68,69]. Trophic factors are helper molecules that allow neurons to develop and maintain connections with their neighbors. They help establish the connection and allow motor neurons to communicate with their target cell, skeletal muscle cells. They help maintain and support motor neurons and also aid in repairing damaged motor neurons back to a healthy condition[23]. However, although animal trials were successful in proving the healing properties of these factors, human trials did not produce the same success and need to be further researched due to the factors' inability to reach the target cell but large amounts being too harmful.

Another way MSCs help repair damaged motor neurons is through mitochondrial transfer. The use of mitochondrial transfer can aid in tissue regeneration and repair, and can also restore the bioenergetic needs of damaged cells[70]. This transfer primarily occurs through the formation of intracellular nanotubes, gap junctions, and microvesicles for cell-cell

communication. Numerous studies have demonstrated that there has been an upregulation of mitochondrial respiration and ATP levels with a simultaneous reduction of oxidative damage upon the culturing of MSCs with injured tissue, indicative that mitochondrial transfer is an important means by which cellular functions and energies can be restored.

There is also growing evidence to suggest that MSCs have anti-apoptotic effects, which limits the extent of damage to improve tissue healing. Although more research has to be done in this area, exosomes are used to release factors that indirectly have this effect[69]. The secretion of exosomes is an important property of ALS, which allows for the reduction of the effect of pathogenetic mechanisms in order to heal damaged cells. Exosomes have the ability to cross the blood-brain barrier, which is a unique ability and is significant for neurological disorder therapeutics/treatment. They secrete therapeutic factors like cytokines that limit inflammation as well as trophic factors that help maintain brain and spinal cord connectivity to muscle cells. Additionally, studies have shown that MSC derived exosomes mediate angiogenesis[8], and this enhancement of angiogenesis and myogenesis promotes muscle regeneration, which is crucial for ALS treatment in which the motor neurons are implicated.

Studies have indicated that MSCs have successfully improved the pathological features and development of ALS and display a high level of therapeutic plasticity[7]. Therefore, MSCs would be a promising cure as it allows for differentiated motor neurons to replace damaged cells while also providing mechanisms for the damaged cells to repair themselves.

8. Practical Applications

There have been numerous studies conducted regarding MSCs and their applications as a therapeutic for ALS, many of which have offered a greater insight into potential applications of MSCs in a clinical setting. Clinical trials have indicated that MSC cells, upon their transplantation, are capable of inducing a substantial upregulation of neurotrophic factors, some of which include the glial-derived neurotrophic factor and the basic fibroblast growth factor. Neurotrophic factors are crucial with respect to the consideration of potential treatments for ALS, since it has been

demonstrated that they prolong motor neuron survival in ALS. A unified delivery of various neurotrophic factors has been shown to have a synergistic effect on the pathogenesis of ALS itself, and MSCs induced with neurotrophic growth factors are demonstrated to display protective effects in many neurodegenerative disease models[71].

In a similar clinical trial, a medium based MSC induction process was devised in which MSCs were induced with neurotrophic factors, which possessed the ability of an enhanced secretion of GDNF, VEGF (vascular endothelial growth factor), the hepatocyte growth factor, and brain-derived neurotrophic factors[71]. Upon the transplantation of these neurotrophic factor induced MSCs, no significant adverse effects were noted, and a higher secretion of neurotrophic factors was observed, which directly correlated with a substantial improvement in the monthly rate of decline of ALS pathogenesis. Similarly, it has been found that when MSCs have been cocultured with healthy tissues, there is a reduction in oxidative damage, an upregulation of mitochondrial respiration (indicative of proper cellular function and mitochondrial transfer), and an upregulation of ATP levels[70]. In general, though, the practical and clinical applications of MSCs are attributed to their differentiation plasticity, their capability of the secretion of bioactive compounds as dependent on the microenvironmental condition (playing a role in immunomodulation, reduction of inflammation, and regeneration), their ability to migrate to sites of injured tissue upon intravenous injection, and their immunomodulatory capacities[72].

9. Future Directions

MSCs do present a few challenges with regard to their transplantation and application as a reliable disease therapeutic that need to be further researched before use. For example, challenges can arise in the characterization of MSCs, because after they are isolated and selected, it must be ensured that the MSCs are pure, active, undifferentiated, and functional for their clinical application. In addition, when MSCs are utilized in cell delivery treatments such as in neurodegenerative disorders, it must be ensured that the microenvironment of the host meets the requirements of the specific type of cell[56]. Also, although MSC therapies are

generally considered to be safe as documented in various clinical studies, the utilization of a relatively large number of living MSCs carries certain risks, such as occlusion within arterioles, capillaries, and venules, a transformation of transplanted cells into inappropriate cell types such as those displaying malignancies, and proarrhythmia. Similarly, the transplantation of living cells with the ability to replicate is inherently high-risk, because the grafted cells cannot be completely removed if there is an adverse response to treatment or if the disease is ultimately resolved[59].

Moreover, it has been demonstrated that the functioning of MSCs declines with age, and this degradation of function can be implicated in the loss of tissue integrity and homeostasis[8]. Interestingly, the functional activity of MSCs typically declines during and as a consequence of cell senescence - a mechanism through which the aging of a cell occurs and cellular replication ceases, with an absence of cell death[73]. This can have a significant adverse impact on MSC function when utilized for the regeneration of injured tissue and therefore, strategies to prevent or delay senescence must be evaluated in order to yield a longer beneficial impact and increased MSC quality[8].

It is relevant to note that a comprehensive study of the mechanisms of differentiation of stem cells can aid in the current understanding of how the brain and spinal cord can be individually targeted to stimulate their repair[74]. Furthermore, the specific pathogenetic mechanisms of exosomes, and their role in therapeutic applications through their capacity of cellular repair should be further examined. The advantages of MSC derived exosomes/extracellular vesicles (EV) have been elucidated in this paper; for instance, although these EVs have demonstrated beneficial effects in the treatment of malignancies, tumor cells through EVs have the potency to function as either tumor promoters or suppressors. Specifically, MSC-derived exosomes have been reported to be involved with tumor growth, angiogenesis, and metastasis. However, there does exist a discrepancy between the behaviors of MSC exosomes, which may be associated with the sources of MSCs, genotypic characteristics of tumors, and the stages of tumor growth[8]. Thus, the side effects and implications of MSC-derived EVs must be further investigated.

Most significantly, the protocols for inducing cellular differentiation into MSCs cannot be directly incorporated within human beings due to their incorporation of toxic chemicals[75]. This warrants further study into the development of induction methods that can be safely executed within patients. Overall, though, the mechanisms of MSCs, in addition to their capacity as a universal therapeutic within a clinical setting for ALS and similar degenerative diseases needs to be examined to a greater extent, in order to gain an increased understanding of the current state and application potential of MSCs, as well as of methods that could be implemented to circumvent the potential risks associated with the administration of MSCs.

10. Conclusion

Amyotrophic lateral sclerosis is a rare and progressive neurodegenerative disease with no cure to date. While the causes of ALS remains unclear, current theories for ALS pathogenesis include mitochondrial damage, glutamate excitotoxicity, neuroinflammation, and oxidative stress. Existing treatments to slow the degeneration of motor neurons and extend survival rates and/or time to tracheostomy include therapeutics like riluzole and edaravone, gene therapy to target

ALS-related gene mutations, and astrocyte transplantations to reverse neurotoxicity in the CNS. However, the mechanisms for these therapies are also not well-understood, and they are not a long-term solution. They mainly target one or two pathogenetic mechanisms or do not provide a solution to treat all ALS cases, so MSC therapy may be the answer to a cure for ALS. Mesenchymal stem cells have been shown to be a promising therapeutic for ALS, based on preclinical and clinical trials, due to their ability to stimulate tissue repair by differentiating into muscle cells as well as providing for means to repair damaged tissue. Challenges associated with MSC therapies such as the high risk and irreversibility deem it essential for further research on the administration of MSC treatments for ALS.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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