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Stem Cell Based Therapies for Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral Sclerosis, or ALS, is a rare neurodegenerative disease. Due to the loss of motor neurons, symptoms of ALS usually involve the progressive decline in motor function. ALS has a variable presentation in patients, making identifying the specific cause of disease quite difficult. Riluzole is currently the only FDA approved medication that reduces the rate of motor function decline and extends the lifespan of patients. The proliferation and differentiation properties of stem cells have opened the doors to new therapies. Mesenchymal stem cells have been selected for their ethicality, safety, and neuroprotective abilities. Through their ability to release trophic and pro-inflammatory cytokines, minimize the reactivity of glial cells, and promote a stable neuronal environment, MSC are a promising therapeutic mechanism for treating ALS. Both preclinical trials, on $SOD1^{G93A}$ rodents, and clinical trials have shown that MSC treatments reduce the rate of disease progression and stabilize the neuronal environment in ALS patients. However, few results indicate that MSC therapies have successfully extended survivability in individuals with ALS. Researchers are hopeful in improving MSC treatments, but further knowledge pertaining to most beneficial method of delivery, cell dose, and location of obtainment is necessary.

Keywords

Stem cell; Therapy; Amyotrophic lateral sclerosis ALS.

Introduction

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is a motor neuron disease [1]. Characterized by the progressive degeneration of motor neurons, ALS is termed as a neurodegenerative disorder. Early symptoms usually begin with muscular weakness and rapidly progress to respiratory insufficiencies, which is the main cause of death for patients with ALS. After diagnosis, ALS patients are projected to have between three to five years before the disease become fatal [2]. Onset of ALS is termed

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as bulbar-onset, beginning with dysphagia and dysarthria, or spinal/limb-onset, starting with muscular dysfunction. Individuals with an ALS diagnosis are grouped into familial ALS (FALS) or sporadic ALS, based on the heritability of their illness [2]. ALS is a heterogenous disease, as the presentation, prognosis, symptoms, and pathophysiology differ between individuals, which makes identifying the definite cause of disease difficult [1]. The dying-back axonopathy theory of motor neuron is growing popularity to describe a potential course of disease for ALS. This theory states that motor deterioration originates in at peripheral neuromuscular junction and progresses towards the spinal cord [3]. Numerous cellular mechanisms have been suggested as the basis of this disease, such as dysregulation of protein and RNA homeostasis and excitotoxicity. In the process of diagnosis tests such as nerve conduction studies, Electromyogram (EMG), and Magnetic Resonance Imaging (MRI) help rule out other neurodegenerative disorders with similar presentation and assist in concluding a definite diagnosis [1]. While there is no cure for ALS, therapies such as physical therapy, occupational therapy, and nutritional assistance help patients manage their quality of life [2]. There is currently very few FDA approved pharmaceutical treatments. Riluzole is the only medication available to ALS patients that has shown positive results in decreasing the rate of muscular degeneration progression and extending survival by a few months [2]. With the increasing popularity of stem cell therapies, it is suggested that stem cell transplantation serves as a potentially viable treatment for neurological and neurodegenerative disorders, such as ALS [4]. Stem cell transplants have shown results of slowing down motor neuron deterioration. In the future, there is hope that stem cell therapies will be beneficial in neural regeneration [4].

Clinical Presentation

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disease that results in the degeneration of both upper and lower motor neurons. This degeneration leads to progressive motor dysfunction, which results in muscle weakness. With a wide array of clinical presentations, sites of on-sets, and symptoms, ALS is a complex disease. A signature feature of ALS is that upper and lower motor neuron symptoms are present simultaneously [1]. Symptoms of upper motor neuron dysfunction include spasticity and weakness. Lower motor neuron dysfunction symptoms are defined as atrophy and cramps. In addition to motor symptoms, some patients develop cognitive and behavioral abnormalities. About 30-50% of patients with ALS experience cognitive and behavioral changes, such as apathy, loss of language fluency and other executive functions, in the early stages of disease [5]. In a study of 146 ALS patients, 24% of patients showed evidence of cognitive decline in later stages of their disease while presenting cognitively normal at the time of onset. Individuals manifesting cognitive and behavioral dysfunction during their illness showed more rapid deterioration and shorter survival time than those whose cognitive status remained stagnant [5]. A portion of cohorts also developed frontotemporal dementia (FTD) [6]. About 60% of patients experience spinal or limb onset, which is the emergence of symptoms in the upper or lower limbs and usually appears asymmetrically [2]. The other large portion of patients experience bulbar onset beginning with symptoms of dysarthria followed by dysphagia, which eventually spreads to the limbs [1]. Research has correlated bulbar onset with a higher risk of developing FTD [7]. Bulbar onset is also associated with unexplained weight loss, giving evidence to a possible association of hypermetabolism with ALS [2, 7]. Spinal onset, bulbar onset, and respiratory onset are both considered classical phenotypes of ALS, as onset begins with both upper and lower motor neuron degeneration. Non-classical clinical phenotypes such as flail leg, flail arm and hemiplegic onset with symptoms originating in

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either the upper or lower motor neurons [6,7]. As ALS advances, the patient will develop symptoms of both upper and lower neuron decay [2].

Gross pathological characteristics of ALS include deterioration of skeletal muscles and the motor cortex. The pyramidal tracts experience sclerosis and pallor, which are common due to the degeneration of motor neurons and loss of myelination [1]. These pyramidal tracts are efferent nerve fibers running from the cerebral cortex to the brainstem and spinal cord and function in controlling voluntary muscle movement. Thinning of the ventral root of the spinal nerves, which transmits motor signals from the central nervous system to the skeletal muscles, is also common to see in ALS patients [2]. The combinations of damages of the ventral root and pyramidal tract directly cause the characteristic motor symptoms of ALS such as a spasticity and weakness. Dysphagia and dysarthria, on the other hand, are correlated to the atrophy and thinning of the twelfth cranial nerve, the hypoglossal nerve, which directs movements of the tongue.

Epidemiology

In the United States of America, data from the National ALS Registry, from 2012-2019, showed an overall incidence of 1.44 per 1,000,000 persons. Incidence appeared greater in White individuals compared to those of other races, in the United States. When plotted on a map, northern states appeared to have a greater incidence of disease than southern states, but this could be attributed to a greater proportion of White residents [8]. In a meta-analysis, the standardized global incidence of ALS was found to be 1.68 per 100,000 persons but varies by geographical area. Incidence by sex and age also varies, with males having a significantly greater incidence than females and individuals between the ages of 60 to790 having a greater incidence than other age ranges [8-10]. Results of incidence studies vary with some evidence showing stable incidence over the past few decades and others showing an increase in incidence. However, ALS remains categorized as a rare disease [6]. The prevalence of disease has been calculated to be about 5.2 per 100,000 persons but is expected to rise due to increased life expectancy and general increase of the aging individual's [6, 9].

Genetic and environmental

ALS is categorized into subtypes based on heritability. Familial ALS (FALS) is inherited, usually through an autosomal dominant pattern, and accounts for about 10% of ALS diagnoses. Research showed that FALS was distinguished by genetic pleiotropy, in which a single gene can produce multiple phenotypes, and <50% penetrance, which suggests that a genotype does not always present the same phenotype. The other 90% cases are categorized as sporadic ALS, and the cause of disease is unknown [2]. However, evidence has indicated that there is a possibility of oligogenic and polygenetic inheritance for individuals with sporadic ALS [2, 6]. ALS is a heterogeneous disease, which describes the wide array of phenotypes, genotypes, onsets, and courses of disease between individuals diagnosed with ALS. The diversity of this illness makes it extremely difficult to identify the cause of disease. It is suspected that individuals with ALS carry multiple rare variant genetic mutations and, over time, exposure to environmental factors promote disease expression.

Epidemiology studies have suggested that toxins, smoking, physical activity, body mass index (BMI), and blood lipid level might be risk factors for the development of ALS [2, 9]. Due to error and bias present in these studies, the reliability of this data is low. To attempt to identify genetic predispositions genome

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wide association studies (GWAS) were conducted and over thirty mutant genes were identified in numerous cellular mechanisms that have been linked to the development of ALS.

Pathogenesis & pathophysiology

Many cellular mechanisms have been associated with the multifactorial pathogenesis of ALS, through the agency of genetic variants. Over 50 genes have been identified as part of these various pathways. Genetic mutations in SOD1, TDP-43, C9orf72, and FUS are seen in almost 70% of cases of familial ALS [2, 11, 12]. Many of these mutations are involved in multiple interactions and mechanisms of pathogenesis [12]. A multitude of other genetic variants can be identified in both FALS and sporadic ALS. However, individuals that display these genotypes do not always express the phenotype of disease [9].

Impaired protein homeostasis as a pathophysiology of ALS, involves compromising protein turnover. SOD1, C9orf72, and TDP-43mutations have been correlated with the dysregulation of chaperone proteins, responsible for protein folding, refolding, and the disassembly of protein aggregates, and a decrease or damage in the expression of proteasomes, such as the ubiquitin-proteasome system (UPS), which break down misfolded or dysfunctional proteins [12]. These aggregates are also present in other neurodegenerative disorders such as Alzheimer disease and Parkinson disease [2]. Mutations in SOD1 have been correlated to the decreased function of chaperone proteins and proteasomes results in misfolded protein bodies that accumulate within the neural tissue. This accumulation and assemblage of protein inclusions in motor neurons is a main neurological indicator of ALS. TDP-43 mutations may be involved with the assemblage of cytoplasmic protein inclusions through inducing neurotoxicity resulting in UPS damage. The dipeptide repeat protein mutation of the C9orf72 gene decreases the degradation of toxic proteins. The product of the translation of the RNA segment containing the repeat sequence is cytotoxic and causes impairment to the UPS system and over autophagy causing aggregation of misfolded proteins usually located outside of the motor regions of the nervous system. The C9orf72 mutations have also been associated with extra-motor symptoms such as cognitive and behavioral impairments [2, 12].

Disruption in RNA synthesis, transport, and stability, is another proposed source for ALS pathogenesis. RNA-binding proteins coded by genes TDP-43 and FUS are normally localized within the nucleus. With FUS mutations, the FUS protein is misallocated to the cytoplasm instead of the nucleus causing an immunoreaction and protein accusations. TDP-43 mutations cause abnormal RNA splicing resulting in mRNA instability. This can either cause a gain of function resulting in toxic protein accumulation or loss of function causing neuronal depletion [12].

Excitotoxicity of motor neurons is another proposed pathway of pathogenesis of ALS, which is the result of dysregulation of glutamate transporters. Excitatory Amino Acid Transports (EAAT 1 and EAAT2) and their rodent homologues, GLAST and GLT-1) are primary transporters of glutamate. Located on both astrocytes and neurons, these transporters help maintain an extracellular and intracellular homeostasis of glutamate. Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) is the glutamate receptor that mediates calcium influx through subunits. AMPAR is a tetramer and the GluA1 subunit is responsible for driving calcium permeable receptors, while GluA2 is responsible for impermeable calcium receptor activation [2, 12]. This produces an accumulation in glutamate in the synapse of motor neurons, which leads to neural toxicity. SOD1 mutants upregulate caspase-3 post-translational cleavage of EAAT2,

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impairing the transporter's ability to uptake glutamate resulting in excitotoxicity. C9orf72 mutants that lead to the toxic dipeptide repeat sequence increases expression of GluA1 and increases Ca+2 permeability causing excitotoxicity [12]. Excitotoxicity has been identified in both rodents and humans with ALS, which leads to the hypothesis that this is a common mechanism of ALS. Mutations have also been seen to cause phenotypic changes in glial cells, altering their functions. These reactive glial cells appear to produce pro-inflammatory cytokines, excessive neuronal pruning, and other activities resulting in motor neuron death [2].

Other pathways also considered in the pathogenesis of ALS are mitochondrial dysfunction, axon dysfunction, impaired DNA repair, and endosomal transport. It is significant to note that the effects of ALS are not localized to just the neural cells but also are dependent on the extracellular matrix and glial cells. Presence of toxicity and external masses, cause harm to the functionality of the central nervous system, as well. Due to the vast amount of diversity between the genotypes and phenotypes of individuals with ALS, no definite conclusion has been drawn about pathogenesis [2].

Progression & prognosis

The initial appearance of symptoms gives insight into the pattern symptoms will advance, which is significant in determining the course of disease. The progressive nature of this disease is linear, causing symptoms to rapidly increase in severity over time [1]. Early symptoms of weakness, eventually result in paralysis, leading to respiratory insufficiencies or dyspnea, which result in death [11]. The process of progression of symptoms and overall prognosis of ALS varies between patients. Bulbar-onset and respiratory-onset have been correlated with a shorter survival time. Other poor prognostic factors include symptoms of cognitive decline, such as FTD, and hypermetabolism, resulting in weight loss. Algorithms that measure prognosis have been generated from population based and clinical based trials, but variability is constantly questioned leading to revisions and the development of new prognostic diameters [2].

Most clinicians and researchers utilize the revised ALS functional rating scale (ALSFRS-R), which tracks the progression of disease through accessing the patients' functional abilities. ALSFRS-R is a 48-point scaling system rating the 12 aspects of physical functionality, with a higher score representing better functionality [13]. Professionals favor this system due to its simplicity and the ability to translate a patient's numerical scores into stages of disease [13]. However, this scoring system has limitations that raise questions about its validity. The total raw scores of patients evaluated with ALSFRS-R are difficult to compare as scores are subject to variability. A one quantity of change for an individual can correspond to a different level of functional change in another individual based on their different starting points of disease and overall range of change, making reliable comparison between subjects difficult [13]. ALSFRS-R, also, does not account for improvement in symptoms or medical assistance such as ventilation that could affect advancement of symptoms [7]. Forced vital capacity (FVC) is another common parameter of predictive measurement of disease progression. Often used alongside ALSFRS-R FVC, is used to measure respiratory function.

The staging systems provide categories to define where an individual generally is in terms of disease progression. The King's staging system stems from the appearance of respiratory or digestive failure and

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the number of regions of the body affected. The Milano-Torino staging (MITOS) system uses the ALSFRS-R responses in four domains, respiratory, fine motor, gross motor, and bulbar, to categorize patients in one of six stages. The King's staging system is better used for earlier stages of disease while the MITOS system is best used for later stages of disease, making both systems complementary [1, 7]. Though neither of the staging systems are used in clinical practice both have been termed useful in certain clinical trials [7].

Diagnosis, screening, & imaging

The diagnostic process for ALS is complex due to the disease's heterogeneous presentation and a lack of distinct biomarkers. A key step in the process of diagnosing ALS is ruling out all other neurodegenerative and motor neuron disease with manifestations that clinically overlap. With no singular diagnostic test, diagnosing ALS primarily relies on the identification of decline of motor function, the presence of motor neuron degeneration anatomically, and advancement of dysfunction over time [9]. Electrodiagnostic testing, including electromyography (EMG) and nerve conduction studies, are vital in detecting amyotrophic lateral sclerosis in patients and ruling out other similarly presenting diseases. Magnetic Resonance Imaging (MRI) is also significant in eliminating other possible diseases.

Motor nerve conduction studies are performed alongside EMGs to measure action potentials travelling from nerves to muscles. Normal conduction is usually found at the onset of disease. As disease progresses the studies may show a mild decrease in conduction velocity and compound muscle action potential (CMAP) and slightly extended distal motor latency. While axonal loss is a source of these results, decreased CMAP can also be due to demyelination causing conduction blocks. ALS has an axonal pathology, so indications of myelin pathology reflect results are related to another disease [14]. Sensory nerve conduction studies should be completely normal for a diagnosis of ALS, as the dorsal root ganglion is unaffected [14]. Abnormal results indicate possible other sources of symptoms.

EMGs are used to detect neuromuscular abnormalities through measuring electrical signals of the muscle fibers and reliably dismissing conditions with similar manifestations as ALS. Needle electrodes, specifically, help detect lesions of lower motor neurons. Distal muscles are usually affected by motor neuron lesions first, so it is crucial to examine the proximal muscle. A reduced number of F-waves indicate possible conduction blocks in an otherwise normal muscle [14]. EMGs measure denervation through changes in the motor unit action potential (MUAP) within nerves. Acute denervation can be seen as fasciculations, fibrillation and positive sharp waves, but while seen in early stages of ALS these fasciculations are not ALS specific [1,14]. Chronic denervation is indicated by prolonged MUAP duration, due to a decrease in motor units available. The unaffected anterior horn cells attempt to reinnervate muscle fibers indicated by large amplitude MUAP, as nerves integrate more fibers than normal [1,9]. EMG is also used to measure the motor unit number estimation (MUNE). However, most studies use the motor unit number index (MUNIX), which assess muscle loss using both the CMAP and amplitude of MUAP [14]. Criteria for ALS diagnosis with an EMG are acute or chronic denervation found in a minimum of three spinal levels. Alternatively, if denervation is not apparent in the spine, ALS can be diagnosed if the EMG identifies denervation in three extremities, with each extremity involving two muscles and two different nerve roots [1,9].

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The El Escorial criteria is the standard diagnosing ALS and provides insight into prognosis. The original El Escorial criteria were revised to increase sensitivity of electrodiagnostic and standardizes ALS into categories of diagnostic certainty [2,14]. This criteria states that the degeneration of both upper and lower motor neurons must be proven in at least one the four anatomical motor regions: bulbar, cervical, thoracic, and lumbosacral and there must be evidence to progressive spread of symptoms within region or to other regions, without the evidence of other disease or explanation for these symptoms [9]. The revised El Escorial criteria requires the presence of upper motor neuron degenerative signs by clinical examination and lower motor neuron degeneration signs by clinical, neuropathological, or electrodiagnostic examination [6]. To acknowledge lower motor neuron lesions, fasciculation, and other EMG evidence, Awaji-Shima criteria were developed. The Awaji criteria allow for earlier diagnosis and decrease the chance of a false positive diagnosis [9].

MRI allows assessment of neural tissues using magnets and radio waves to produce 3D images. These images do not directly identify ALS within patients. Most ALS patients present with normal MRI results. However, if findings are present, they might be identified by a higher intensity within the corticospinal portion of the pyramidal tract and corpus callosum [1,7]. Studies have found decreased signals within the precentral gyrus, which is neuronal loss in the primary motor cortex [1]. Diffusion tensor imaging (DTI) and diffusion weighted imaging (DWI) are MRI techniques that help tract white matter changes that are present in early stages of ALS [7].

Treatments

There is no cure for ALS; treatments consist of managing symptoms to prolong the patient's quality of life. A combination of physical therapy, occupational therapy, speech therapy, and nutritional support are all common forms of treatment for ALS patients in addition to medications [15]. Riluzole was the first FDA-approved pharmaceutical drug for ALS and has been used as a treatment since 1995 [16]. The mechanism of action of Riluzole is suspected to reduce glutamate-induced excitotoxicity through inhibiting glutamate release. Riluzole is most effective at a dose of 50mg twice a day and may be increased to 100mg twice a day depending on patients' clinical reaction [15]. In an analysis of 15 clinical studies, 8 of these studies found that ALS patients treated with riluzole had significantly longer median survival than untreated patients, ranging from 6 to 19 months [16]. In 2017, edaravone became the second FDA-approved drug for ALS treatment. Edaravone reduces oxidative stress by acting as a free radical scavenger and assists in reducing mutant SOD1 protein accumulation [15]. In a randomized, double-blind, placebo-controlled phase III study, the patients treated with edaravone demonstrated a reduction in their rate of motor function decline and neuron degeneration [15]. Edaravone is administered intravenously at a concentration of 30mg of edaravone and 100mL of isotonic solution. The usual treatment cycle included 60mg of edaravone given over a span of 60 minutes daily for 2 weeks followed by a 2week drug free period [15]. Tofersen is another FDA-approved drug used to treat the SOD1 mutation. In 28week, clinical trials, treatment with tofersen was seen to reduce the amount of SOD1 protein in patients' cerebral spinal fluid and plasma but showed no change in the rate of functional decline. However, longer term studies showed sustained decrease in the SOD1 protein and a reduced function decline, associated with earlier implementation of treatment [17]. Tofersen is administered intrathecally. The three initial doses (100mg) were given in intervals of 14 days followed by one maintenance dose every 28 days [15].

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Stem Cell Therapy

Stem cells are immature precursor cells that are the basis of all the cells in the body. To be categorized as a stem cell, a cell must encompass the ability to regenerate and produce an identical progeny and be able to differentiate into specialized cells of the body. Generally, stem cells can be categorized based on their differentiation potential. Totipotent stem cells can differentiate into the three embryonic tissues and extraembryonic tissue. A zygote is the only example of a totipotent stem cell. Pluripotent stem cells can differentiate into all adult cell types, while multipotent stem cells can only differentiate into numerous cells within a specific cell lineage [18]. Stem cells also can be categorized by their origin. Human Embryonic Stem Cells (hESCs) are pluripotent cells derived from the inner cell mass of a human blastocyst. However, hESCs can be harvested within a laboratory, as well. After these stem cells have been obtained, under specific conditions, they can indefinitely propagate in vitro, preserving their ability to differentiate into numerous types of different specialized cells. However, research on hESCs is controversial due to the destruction of embryos from which the cells are obtained. Also, the rapid proliferation of ESCs increases the possibility of the formation of malignant growth when used in treatments [19]. Adult Stem Cells are multipotent cells that originate from various bodily structures and locations after embryonic development. Some examples of these stem cells are Mesenchymal Stem Cells (MSCs), Hematopoietic Stem Cells (HSCs), and Neural Stem Cells (NSCs). Adult stem cells are favored over other forms of stem cells for research and therapeutic methods because they can be obtained safely, eliminating the ethical concerns that follow the use of ESCs [19]. In addition, they are isolated and expanded much easier than other forms of stem cells. Adult stem cells' limited replication time, compared to other stem cells, decreases the possibility of malignant growth. Induced Pluripotent Stem Cells (iPSCs) are derived from the reprogramming of adult somatic cells. While iPSC shares many characteristics with ESCs, such as their wide differentiation capabilities, these somatic cells are obtained ethically and safely from multiple areas of the adult body [19]. These cells undergo genetic alterations to integrate four factors found in ESCs that produce pluripotency. iPSCs allow for the therapeutic potential of autologous transplantation, thereby reducing the possibility of immune rejection [19].

Because of their self-renewal capabilities, many researchers have studied the application of stem cells in regenerative therapies. For neurological diseases, such as ALS, Alzheimer's disease, spinal cord injuries, and multiple sclerosis, discoveries of stem cell-based therapies have opened the doors to the possibility of cell regeneration and further new emergent therapy options [19]. The success of stem cell-based therapies is dependent on the stem cells abilities to secrete growth factors, regenerate and differentiate into neural cells, and the success of forming a connection between transplanted cells and host cells [20]. Evidence through preclinical studies has shown that both ESCs and iPSCs transplantation could result in the formation of new neural tissue connections and increase patient survival time, in SOD1 animal models [19]. The discovery of iPSC has, also, led to promising possibilities of clinical success in treating neurodegenerative disease by allowing for autologous transplantation [20]. However, their utilization is limited due to ethical concerns of obtainment and rejection, as previously stated [18-20]. During preclinical and clinical trials involving MSC infusions, evidence has pointed towards providing protection against neural damage due diseases such as ALS [18, 19]. MSCs are suggested to be the ideal treatment option for neurodegenerative disorders.

Mesenchymal stem cells are adult multipotent stem cells that can be easily and ethically isolated from either an adult somatic source, such as bone marrow, peripheral blood and adipose tissue, or from a fetal source, such as the amniotic membrane and the umbilical cord. MSCs must possess the ability to proliferate by mitotic cell division, to self-renew over long periods of time, and a pluripotent ability to differentiate into a variety of cell-lineages, according to the International Society of Cellular Therapy (ISCT) [21]. These cells are not immunogenic because they lack the expression of major histocompatibility complexes (MHC) which eliminates the need for an immunosuppressive drug injection before treatment. In addition, MSCs migratory capabilities permit these cells to travel throughout the body to damage sites to assist in tissue regeneration. MSCs are studied as a probable therapeutic approach for ALS mainly due to their ability to differentiate into functional neural-like cells and to repair the extracellular matrix surrounding these neural cells through the secretion of trophic and growth factors and removing toxic substances, therefore decreasing inflammation. In turn, they promote the repair of neuron-supporting cells and neuron cells, further supporting the repair of neural connections and promoting the formation of secondary brain pathways to improve overall motor function in ALS patients [21]. These cells remain uncommitted until they receive a signal to differentiate into a specific specialized cell. Despite the current findings, further research is necessary to determine the most effective type of stem cell for treatments and dosage, as well as mechanism of action.

Discussion

With the astounding abilities of plasticity and wide range of differentiation potentials of stem cells, stem cell therapy provides a potentially beneficial treatment for neurodegenerative diseases. Through their regenerative capabilities and ability to differentiate into numerous cell types, the popularity of stem cell transplantations as a treatment for ALS has increased. Early preclinical studies with animal models presented evidence that supported the functional, cognitive, and physical benefits of the implantation of ESCs and iPSCs in disorders of motor neuron dysfunction [20, 22, 23]. Recent regulations put in place to address the ethical concerns of using certain types of stem cells has redirected research and trials to focus on the use of MSCs as an alternative. MSCs can be easily and ethically isolated from either an adult somatic source, such as bone marrow, peripheral blood and adipose tissue, or from a fetal source, such as the amniotic membrane and the umbilical cord. These cells are not immunogenic because they lack the expression of MHC, which eliminates the need for an immunosuppressive drug injection before treatment. In addition, MSCs migratory capabilities permit these cells to travel throughout the body to damage sites to assist in tissue regeneration [21]. MSCs are favored as a therapeutic approach for ALS mainly due to their ability to differentiate into functional neural-like cells and to repair the extracellular matrix surrounding these neural cells through the secretion of trophic and growth factors and removing toxic substances, therefore decreasing inflammation. In turn, they promote the repair of neuron-supporting cells and neuron cells, further supporting the repair of neural connections and promoting the formation of secondary brain pathways to improve overall motor function in ALS patients [21].

Preclinical trials

Preclinical studies surrounding the potential use of MSCs therapies for the treatment of ALS predominantly depends on the use of transgenic rodent models. Genetic mutations known to play a large part in the pathology and progression of this disease, usually SOD1G93A, are introduced into these

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rodents to replicate the course of disease of ALS [21]. When using mice or rat models for these trials, it is necessary to preform immunosuppressive procedures as the transplanted cells are obtained from humans. Multiple routes of transplantation have been tested on these rodents, such as intrathecal, intravenous, intramuscular, and intracerebral, and all methods have presented promising results [20]. Different trials have utilized MSCs obtained from various location throughout the human body. Specifically, results from three trials containing MSCs derived from different sources and delivered differently will be compiled into this metanalysis. In one preclinical experiment, symptom-presenting SOD1G93A rats received a single intrathecal delivery of human MSCs derived from human bone marrow (BM-MSCs) at a dose of 5 x 105 (5 uppercase) [20]. An intrathecal injection refers to the administration of substance directly into an organism's cerebrospinal fluid, in this case directly into the brain but in humans is usually administered through a lumbar puncture. Two wildtype groups were also created, one injected with BM-MSCs and the other with the vehicle [20]. Another preclinical study investigated the therapeutic effects of human fetal umbilical cord MSCs (hUC-MSCs) through multiple intracerebral ventricular injection of a dose of 2.5 x 105 (5 uppercase) [24]. The treatment was administered through an intracerebral ventricular injection, which involves the injection of a substance directly into the brain ventricles. The treatment and control groups were further divided for analysis. Transgenic mice receiving an injection every fourteen days, for a total of three injections, were analyzed for histological and biochemical changes. The other subset of transgenic mice was used for motor behavioral tests and received a total of four injections that were administered once every fourteen days [24]. A third preclinical trial used hMSCs derived from a fetal amniotic membrane (hAMSCs) to determine the cells effectiveness as a treatment option for ALS. Through multiple intravenous delivery to the jugular vein, hAMSCs were administered to SOD1G93A mice. Treatment of hAMSCs was delivered through the jugular weight of these mice at ages of 12, 14, and 16 weeks [25]. In all three different preclinical trials, the MSC treated rodents were compared with a group of SOD1G93A transgenic rodents injected with a vehicle (PBS). Both these groups were compared to the wildtype of the rodents to analyze the results. The various of sourced MSCs provides evidence towards the possible therapeutic effects and benefits of deriving these cells from different sources.

Each preclinical study used a variety of tests to determine the effects of MSCs transplantation on the progression of ALS, specifically through observing the behavior of motor function in the symptom presenting SOD1G93A rodents. Trials using BM-MSC, hUC-MSC, and hAMSC transplants into SOD1G93A rodents, conducted grip strength experiments to determine the effects of these stem cells on motor performance throughout the disease. Results from the grip strength test showed that SOD1G93A rats treated with BM-MSCs has a notable improvement of their motor strength at the later stages of disease compared to the vehicle injected rats ($p<0.05$) (**Figure 1**) [20]. However, SOD1G93A mice treated with hUC-MSCs presented a slight but not significant improvement in motor performance (**Figure 2**) [24]. Mice treated with hAMSCs, also, presented significantly higher motor grip strength during disease, which was measured with a PaGE test, than those who received the vehicle (**Figure 3**) ($p<0.05$) [25]. Performance effects of the treatment with BM-MSCs, a locomotor rating scale called Basso-Beattie-Bresnahan (BBB) scoring test was conducted on SOD1G93A rats. Compared to PBS treated SOD1G93A rats, BM-MSC treated SOD1G93A rats showed a significant reduction in the pace of deterioration of motor function (**Figure 1**) ($p<0.05$) [20]. This difference between groups in motor function decline increased as the disease ran its

course. Transgenic mice groups treated with hUC-MSC and hAMSC were assessed through a RotaRod test to test the rodents' latency to fall and coordination of motor activity. Those treated with hAMSC showed significantly greater balance and coordination after the second ($p<0.01$) and third dose ($p<0.001$) of treatment but not the first (**Figure 3**) [25]. On the other hand, hUC-MSC treated mice showed a slight increase in motor performance but not statistically pronounced (**Figure 2**) [24]. The evidence of these investigations suggests that MSCs have the potential to decrease the decline of motor function due to ALS. Intrathecal treatment of BM-MSCs and intravenous treatment of hAMSCs were relevantly more effective in reducing the motor dysfunction throughout disease. In addition, it can be said that the evidence pointing to MSC treatment reducing motor decline could be utilized in future studies to further prolong the onset of disease and further improve survivability.

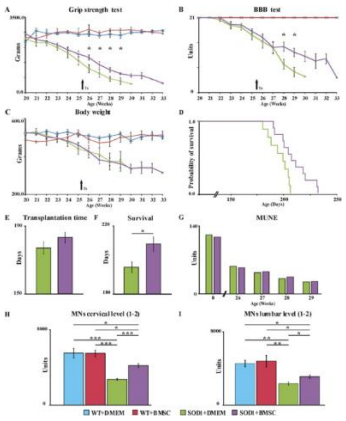

Figure 1: Graphical representations showing that intrathecal delivery of BM-MSCs to $SOD1^{G93A}$ rats is a tolerable treatment that is beneficial in slowing the progression of motor dysfunction and increasing survivability. Statistically significant: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ [20].

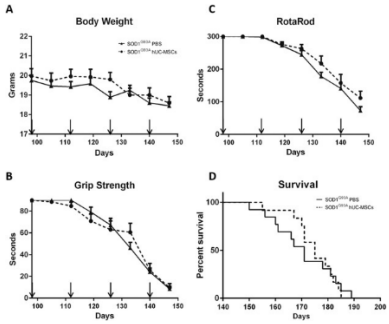

Figure 2: Graphical depiction of intracerebral ventricular delivery of hUC-MSCs SOD1G93A mice. This treatment showed no decline in disease progression and no extension of life. Statistically significant: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ [24].

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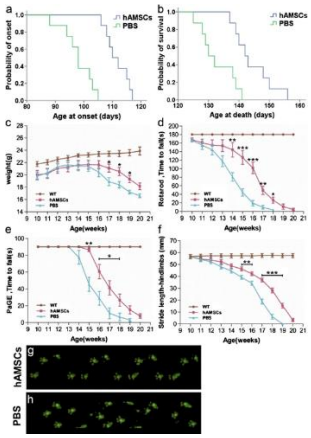

Figure 3: Graphical representation of motor progression of SOD1^{G93A} mice injected with hAMSCs through an intravenous delivery. Motor performance through disease progression was improved, but survivability was not prolonged. Statistically significant: * p<0.05, ** p<0.01, *** p<0.001[25].

Neuroprotective properties of each treatment were investigated through a motor neuron count, because an ALS is characterized by its motor neuron loss. A motor unit estimate study (MUNE) was conducted on the BM-MSC treated SOD1G93A rats' medial gastrocnemius muscle. The MUNE results showed both transgenic groups had a significant decline in motor neurons, but no difference in the amount of reduction between the BM-MSC treated group and the vehicle treated group (**Figure 1**) [20]. Similarly, an immunohistochemistry stain was conducted on the tibialis anterior muscle of hUC-MSC treated mice, to determine the extent of neuromuscular denervation. Transgenic mice groups showed extensive denervation in this muscle, which the treatment of hUC-MSC was ineffective in the reestablishment of these neural connections (**Figure 4**) [24]. Motor neuron loss was detected in the lumbar spinal cord section in both groups SOD1G93A rodents when compared to the wildtype (**Figure 1**) (**Figure 5**) (**Figure 6**) [20, 24, 25]. After death, transgenic mice significantly preserved a higher number of motor neuron in the lumbar spinal cord with the treatment of BM-MSC ($p<0.05$), hUC-MSC ($p<0.05$), and hAMSC ($p<0.01$), when compared to the vehicle treated transgenic groups (**Figure 1**) (**Figure 5**) (**Figure 6**) [20, 24, 25]. BM-MSC treated rats showed considerably more motor neurons rescued in their cervical spinal cord section ($p<0.001$) than their lumbar spinal cord section ($p<0.05$) compared to the vehicle treated group, which suggests that the location of administration influences the success of motor neuron rescue (**Figure 1**) [20]. These results in combination that MSC treatment can rescue motor neurons and minimizing their degradation. However, these motor neurons are unable to reform their connection with the target muscle, which is indicated by the lack of neuromuscular junctions detected in the tibialis anterior muscle (**Figure 4**) [24]. This supports the dying-back axonopathy theory of ALS [20]. This theory states that degeneration begins at the site of neuromuscular junctions in the peripheral nervous system, and the disease progresses in a retrograde direction (distal to proximal) towards the cell body of the motor neuron. Therefore, axonal degeneration occurs before symptoms begin and is followed by neuronal degeneration. It is suspected that this is correlated to the size of the most distal area of the axon [3].

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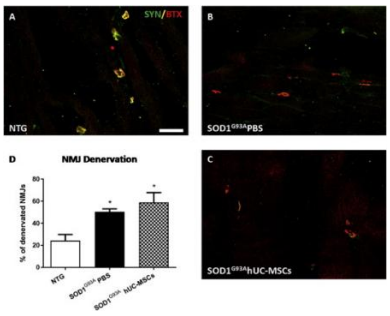

Figure 4: Neuromuscular junction denervation visualization using immunohistochemistry. Section of the tibialis anterior muscle of SOD1^{G93A} mice treated with hUC-MSCs through an intracerebral ventricular delivery was used. No significant renovation was observed because of treatment. Statistically significant: * p<0.05, ** p<0.01, *** p<0.001 [24].

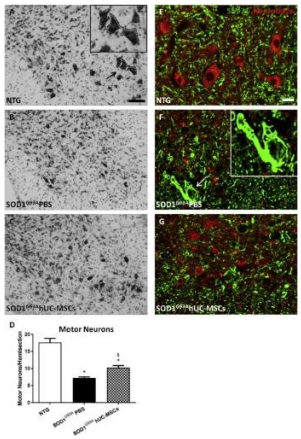

Figure 5: Immunohistochemistry evaluation of the amount of motor neurons presents in the lumbar section of SOD1^{G93A} mice receiving an intracerebral ventricular delivery of hUC-MSCs. Treatment significantly rescued motor neurons. Statistical significance: * =p < 0.0001 vs NTG; § = p < 0.0001 vs SOD1^{G93A} mice injected with vehicle [24].

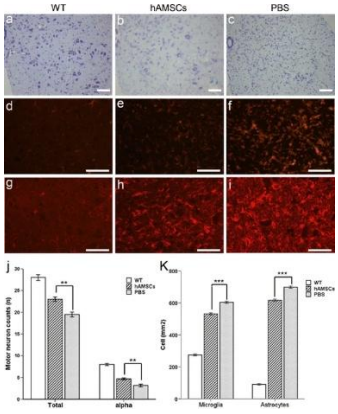

Figure 6: Graphical representation of immunohistochemical results of the detection of motor neurons and the presence of reactive microglia and astrocytes in the lumbar spinal cord section of hAMSC treated SOD1G93A mice through an intravenous delivery. Motor neurons were successfully preserved, and the amount of reactive glial cells was significantly declined with treatment. Statistically significant: * p<0.05, ** p<0.01, *** p<0.001 [25].

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In past studies, it has been suggested that ALS progression and motor neuron loss is due to partly due to mutations resulting in reactive astrocytosis and microgliosis. Microglia and astrocyte cells are glial cells that participate in numerous neurological functions. Mainly microglial cells function as the immune cells of the central nervous system and support the pruning and maturation of the nervous system during development [26]. Astrocytes play a large role in cell communication in the synapse and function in the removal of toxic substances. In ALS, these cells are altered due to mutations which cause them to malfunction, resulting in the release of pro-inflammatory cytokines and neurotoxins, decreasing neurotransmitter uptake, and dysregulating the immune system [26]. It has been hypothesized that MSCs would be effective in targeting these reactive glial cells to reduce motor neuron loss and maintain a functional neural environment. Immunostaining with GFAP (astrocyte specific marker) revealed a surprising increase in the presence of astrocytes in SOD1G93A mice treated with hUC-MSCs than SOD1G93A mice injected with the vehicle ($p<0.0001$) (**Figure 7**) [24]. However, staining with IB1 (microglial specific marker) showed a significant decrease of microglial with the treatment of hUC-MSC, which was expected ($p<0.0001$) (**Figure 8**) [24]. Using the same methods and markers, treatment of hAMSC significantly decreased the numbers of both microglial and astrocytes in treated transgenic mice compared to vehicle transgenic mice ($p<0.001$) (**Figure 6**) [25]. The differences between the results of reducing glial cells between the treatments of hUC-MSCs and hAMSCs is unknown. It could be suggested that these contrasting results could be related to a variation of doses, different types of delivery, or different types of MSCs. It has been stated that intravenous delivery lacks effectiveness due to transplanted cells getting lost in circulation, stuck in peripheral areas, or being unable to pass the blood brain barrier.

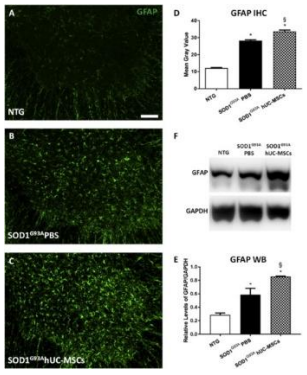

Figure 7: Graphical representation of immunohistochemical results of the detection of reactive astrocytes using the marker GFAB. Astrocyte presents increased in $SOD1^{G93A}$ mice injected with hUC-MSCs. Statistical significance: * =$p < 0.0001$ vs NTG; § = $p < 0.0001$ vs $SOD1^{G93A}$ mice injected with vehicle [24].

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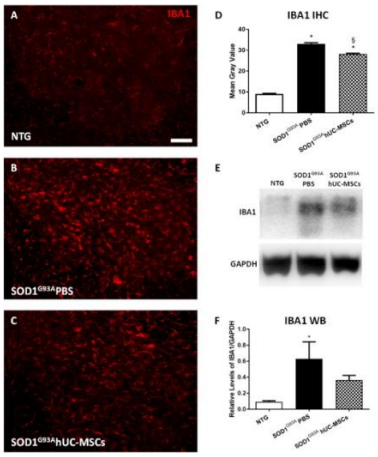

Figure 8: Graphical representation of immunohistochemical results of the detection of reactive microglia cells using the marker IBA1. Microglial presents decreased in SOD1^{G93A} mice injected with hUC-MSCs. Statistical significance: * =p < 0.0001 vs NTG; § = p < 0.0001 vs SOD1^{G93A} mice injected with vehicle [24].

Studies have suggested that decreasing this reactive behavior delays the disease progression of ALS significantly [21]. The damage of these reactive glial cells has been proposed to cause neuronal degeneration through excessive pruning of the nervous system and by creating a toxic neural environment in ALS, which alters neural structures. Perineuronal nets (PNNs) are neural structures that are part of the extracellular matrix. These structures surround neurons and assist in the stabilization of neural synapse and contain neuroprotective capabilities. Abnormal PNN structures are linked to multiple neurological diseases [27]. The visualization of these PNN was quantified through the fluorescent intensity of Wisteria floribunda agglutinin (WFA) stain. Intrathecal treatment with BM-MSCs detectably preserved PNN structures compared to the rats injected with the vehicle at both cervical ($p<0.001$) and lumbar levels ($p<0.01$) (**Figure 9**) [20]. Maintenance of structures was more significant in the cervical regions compared to the lumbar regions in the transgenic treated, suggesting that location of transplant effects is correlated to the effectiveness of structure conservation and suggests effectiveness of MSCs in healing the toxic neuronal environment [20]. The success in the combatting the phenotypes of reactive glial cells in MSC treatment has been proposed to be due their abilities to produce pro-inflammatory cytokines and growth factors [21]. The amounts of pro-inflammatory cytokines, anti-inflammatory cytokines, and growth factors were measured in the lumbar spinal section of SOD1G93A mice treated with hUC-MSCs and BM-MSCs. The hUC-MSC treated SOD1G93A mice proved to display significantly lower levels of proinflammatory cytokines (IL-6 and IL-8), while showing a remarkable increase in anti-inflammatory cytokines (IL-4 and IL-10), and growth factors (IGF-1) in lumbar spinal regions, when compared to the vehicle injected transgenic (**Figure 10**) [24]. However, BM-MSC treated rats had no significant increase in the anti-inflammatory cytokines IL-4 detected in their cerebrospinal fluid [20]. Surprisingly, the treatment of BM-MSCs largely increased the amount of pro-inflammatory cytokines (IL-1α and MCP-1) compared to the wildtypes and vehicle treated transgenic rats (**Figure 11**) [20]. There was only a slight reduction in the pro-inflammatory cytokine TNF-α, while no GM-CSF was detected in either the wildtype or transgenic group treated with BM-MSC (**Figure 11**) [20]. The lack of GM-CSF pro-inflammatory cytokine could be attributed to the direct administration of BM-MSCs into the cerebrospinal fluid. Evidence points to that the production of anti-

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inflammatory cytokines requires multiple administrations of MSCs rather than a single dose. This would more MSCs present in the body to allow to produce a higher concentration of anti-inflammatory properties to combat the pro-inflammatory cytokines already present.

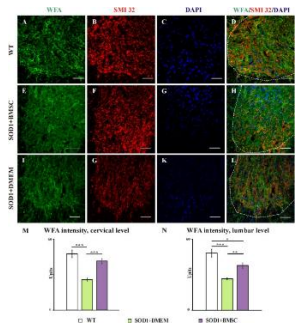

Figure 9: Graphical and immunohistochemical results from the detection of the normal structure of perineuronal nets. Treatment with BM-MSCs significantly preserved PNN structure in transgenic rats. Statistical significance: * p<0.05, ** p<0.01, *** p<0.001 [20].

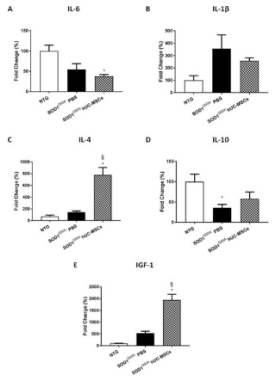

Figure 10: Graphical depiction of the amounts of pro- and anti- inflammatory cytokines and growth factors in transgenic mice receiving the treatment of hUC-MSCs. The number of anti-inflammatory cytokines and growth factors increased, while the amount of pro-inflammatory cytokines decreased. Statistical significance: * =p < 0.0001 vs NTG; § = p < 0.0001 vs SOD1G93A mice injected with vehicle [24].

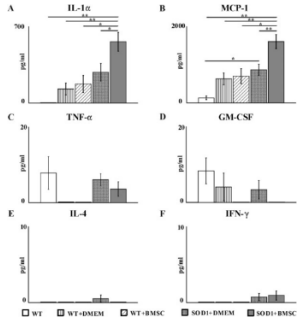

Figure 11: Graphical depiction of the amounts of pro- and anti- inflammatory cytokines and growth factors in transgenic mice receiving the treatment of BM-MSCs. Results between pro-inflammatory cytokines varied, but no increase in anti-inflammatory cytokines was detected [20].

Determining the efficiencies of these treatments is vital in concluding successful a preclinical trial. For all

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three of these studies, body weight was a key indicator of the tolerance to treatment. Treatment of MSCs appeared to assist in maintaining body weight compared to the vehicle treated group. However, compared to the wildtype, ALS rodents loose significant body weight throughout the course of the disease, but this is expected due to muscular atrophy (**Figure 1**) (**Figure 2**) (**Figure 3**) [20] [24] [25]. The biodistribution of transplanted MSCs was also important in determining if the treatment was excepted throughout the body or rejected. For the treatment of BM-MSCs, spinal cord sections were stained with human specific biomarkers for the mitochondria (MtC02) and nuclei (HuNu), but evidence showed that there was no indication of the transplanted cells [20]. Preforming an identical procedure, using rat derived MSCs and rat-specific markers, results still did not produce a positive stain, indicating the lack of stem cell survival was not subjective to rejection [20]. Brain sections were inspected from SOD1G93A mice treated with hUC-MSCs, at various times after each injection. Twenty-four hours after the first injection, high concentrations of MSCs can be seen in the lateral ventricles close to the choroid plexus cells, but after six weeks these clusters decreased dramatically (**Figure 12**) [24]. Brain sections of the transgenic mice were also obtained two weeks after both the second and the third dose of treatment. The hUC-MSCs were still localized to the lateral ventricles, but the more treatments the model received the closer the cell clusters appeared to the hippocampus (**Figure 12**) [24]. Spinal cord section of hAMSC treated mice showed minimal transplanted cells detected in the spinal cord, but none of these cells showed properties of differentiation (**Figure 13**) [25]. Again, these results indicate that a single delivery is not adequate to administer enough MSCs to the body. Though migration is limited in trials of multiple delivery, the MSCs are still present around the site of administration, while in the single delivery no MSCs are present at all. These results cannot be contributed to rejection as the rodents' bodies do not appear to produce weight loss, infection, or adverse reactions. Therefore, the treatment is tolerated the multitude of deliveries is too low.

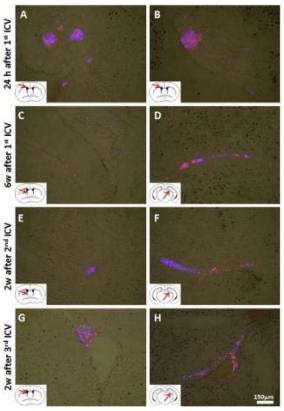

Figure 12: Histological brain sections from transgenic mice sacrificed at various stages after treatment with hUC-MSC. No migration beyond the lateral ventricle of the brain was detected, but with repeated treatment increased amounts of MSCs remained present in the brain [24].

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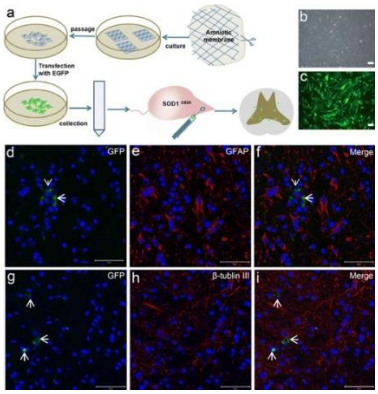

Figure 13: Spinal sections of transgenic mice injected with hAMSCs were stained to determine the migration of transplanted cells. Engrafted cells survived in the lumbar spinal cord section, but migration, proliferation, and differentiation were not detected [25].

Overall, evidence showed that only the treatment of BM-MSC to rodents displaying ALS was significant in increasing lifespan, by extending survivability by 14 days (**Figure 1**) [20]. Increased survivability suggests that intrathecal delivery is the favored method of delivery to produce successful results and that BM-MSCs show the most promising therapeutic results in treatment of ALS. Both treatment with hUC-MSC and hAMSC helped conserve motor neurons in the spine but did not notably elongate the life of the rodent models (**Figure 2**) (**Figure 3**) [24-25].

Clinical Trials

Successfully, preclinical studies on rodents have proved that the transplant of MSCs is a viable treatment option for ALS. Rodent experiments have showed that delivery of MSC through multiple methods is safe for therapies [21]. Recent clinical trials appear to favor the use of autologous BM-MSC in transplantation to avoid the risk of rejection, as they provide clear results of increasing the lifespan of patient in preclinical trials [21]. Clinical trials collectively measured progression of disease using ALSFRS, which is a 48-point scales measuring degree of functionality and in term patient quality of life and FVC, forced vital capacity, which measured the amount of air forcefully exhaled from the lungs after a deep inhalation.

Two phase I clinical trials, conducted simultaneously, investigated the therapeutic effects of autologous BM-MSC transplantation and compared the success and safety of intravenous deliverer (IV) and intrathecal delivery (IT) [28]. Thirty-five patients were screened and evaluated to see if they met the requirements for the trial. Individuals were required to have a definite diagnosis of sporadic ALS, in term of EL Escorial criteria, with more than six months of disease progression [28]. They must be between the ages of twenty-four and sixty years of age, with an ALSFRS score of 24, terming them with 50% of disease progression, and a forced vital capacity (FVC) score of above 40%. FVC score is the measurement of the total volume of air that can be forcefully exhaled from the lung, which indicates overall lung function [28]. All patients presenting other disorder or the use of unauthorized medication were excluded from the trial. Fourteen patients were enrolled in this clinical trial and given 100mg of Riluzole twice a day throughout the study. Patients received a BM-MSC injection of 2 x 106 (6 uppercase) cells/kg either intravenously or intrathecally [28]. Routine clinical assessments 6, 4, 2, and 1 week before the injection and 2, 4, 6 and 12 months after the injection. Assessment including a physical exam, ALSFRS, and FVC [28]. One patient was

lost from both the IV and IT groups, but the other twelve patients completed the trial. It was noted that one IV patients experienced hypotension, while two IT patients experienced headaches and nausea. However, these IT symptoms align with a traditional lumbar puncture, and they resolved themselves [28]. The last follow up, at 12 months, showed no abnormalities in any of the patients' brain or spinal MRIs. Combining the results, both the IV and IT delivery methods for BM-MSCs showed no adverse effects in any of the patients, which solidifies the safety of these delivery methods for treatments. Both the IV and IT groups showed a significant decline in ALSFRS and FVC scores over the 12month follow-up ($p<0.001$), but neither group showed an improvement in scores compared to baseline (**Figure 14**) [28]. Improvements compared to baseline were not detected in preclinical rodent trials and therefore not expected in clinical trials. However, there was no significant difference in the rate of decline between the IT and IV groups for either ALSFRS or FVC, indicating that neither delivery route offered a significant advantage over the other in slowing motor function or respiratory decline (**Figure 14**) [28]. Taking other studies into consideration intrathecal injection directly into the cerebrospinal fluid has benefits to the migration of these transplanted cells and intravenous injection can allow access to various areas through circulation [20] [28]. However, researchers have suggested that IT delivery possibly produces a more viable treatment option as a larger number of cells are able to reach the central nervous system through the direct delivery into the cerebrospinal fluid. IV delivery requires the transplanted cells to circulate throughout the entire circulatory system, possibly losing them in distally located limbs [29]. Both groups showed a continued decline in score indicating disease progression was not hindered but slowed. Therefore, it can be suggested that ALS altered the function of these BM-MSC cells reducing their differentiation and neuroprotective abilities in the body [28].

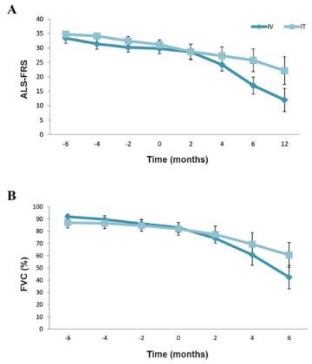

Figure 14: Graphical comparison between ALS patients treated with BM-MSCs through an intrathecal delivery versus an intravenous delivery. Both delivery groups showed a similar decline in motor and respiratory function over 12 months, with no significant difference between routes [28].

A phase I/IIa clinical trial involved the investigation of a single intrathecal delivery of BM-MSCs to patients with ALS [29]. Patients were recruited for this trial with requirements of having definite ALS as defined using EL Escorial criteria and with a FVC less than 70%. Patients were required be between the ages of eighteen and sixty-five, on a stable dose of Riluzole (or none), FVC over 70%, and have a life expectancy of more than two years [29]. Any addition medical concerns or diseases excluded patients from the study. Twenty-six ALS expressing individuals were enrolled and examined 6, 3, and 1 month prior and 3, 6, 9, 12, and 18 months after the BM-MSC administration. All subjects were given 4.5 x 106 (6 uppercase) cells/kg MSCs [29]. Three participants were excluded from results either due to premature death or other medical

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needs. After treatment, 30% of participants experienced moderate headaches, which again is normal after a lumbar puncture. All in all, no adverse reactions were observed in any patients, and no pathological changes were detected in the patients' MRIs after twelve months [29]. These results provide further support that intrathecal delivery of BM-MSCs is a safe procedure that does not cause extreme or severe side effects to patients [29]. Comparing ALSFRS scores of the progression of disease before and after treatment, stabilization in the decline of motor function at three months post treatment is significant ($p<0.02$) and decline become more rapid at six months post treatment, but still significant stabilization is seen ($p<0.05$) (**Figure 15**) [29]. After six months, disease progression resumes at the same rate as before BM-MSC application (**Figure 15**) [29]. Weakness scores in upper and lower limbs were stabilized in majority of the patients for the first three months after administration of BM-MSCs but quickly declined after twelve months [29]. It should be noted that for subjects with stable disease progression prior to the treatment, decline in course of disease is not detectable. Towards the end of disease, progression appears to be no longer linear [29]. Majority of treated individuals presented with a stable FVC value at or above 70% for the first nine months after treatment and 60% remained stable at twelve months post-transplant [29]. These results contradict those of the previous analyzed phase I studies, in which IT and IV groups experienced a significant decline in FVC over the follow-up period [28]. This difference can possibly be attributed to the larger cell dose used in this study, which is almost twice the dose used in the previous trial. Also, patients enrolled in this trial were required to have a significantly higher baseline FVC score (>70%) than those in the previous trial (>40%), and the lower the FVC score, the more extensive the progression of disease is [28] [29]. The evidence gathered in this study indicates that IT BM-MSCs delivery as a safe treatment for ALS and temporarily reduces the progression of disease [29]. Though results are only temporary, the potential for longer term therapeutic effects has been suggested using repeated application of BM-MSCs [29].

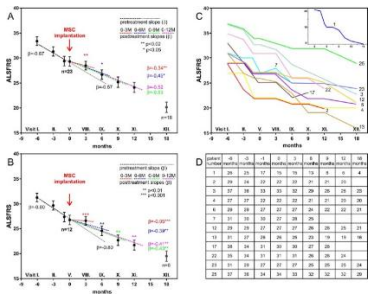

Figure 15: Graphical depiction of motor function decline with the application of BM-MSC through an intrathecal delivery. Patients showed an increased ALSFRS score, indicating decrease in motor decline progression [29].

A third phase II clinical trial was assessed for efficacy and safety. In this trial patients with ALS received two repeated intrathecal injections of autologous BM-MSCs a month apart at a dose of 1 x 106 cells. To participate in the study, patients were required to be diagnosed with clinically probable or definite ALS in terms of the El Escorial criteria. Subjects must have a ALSFRS score between 31-46, FVC above 40%, take a stable dose of 50mg of Riluzole twice a day for at least three months before screening, and onset of disease occurred less than five years prior to this study [30]. A total of 64 randomized patients between the ages of twenty-five and seventy-five were enrolled into the study, 33 of which were treated with MSCs and 31 were a part of the control group. These patients were examined three months prior to the first

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injection and six months following the second injection [30]. The baselines between the treatment and control group were very similar and both groups contained only individuals diagnosed with sporadic ALS. After treatment, few individuals experienced headaches and injection site pain, but there was no difference in these side effects between the treatment and control group [30]. Due to the normality of these symptoms, the treatment was concluded to not cause any severe adverse side effects, and it was termed safe [30]. One patient in the MSC group and three in the control group died before the six month follow up from various reasons, not correlated with treatment. Comparing the ALSFRS-R scores between the treatment and control group, it can be said that the treatment of intrathecal injection of BM-MSCs was effective in slowing down the progression of motor symptoms in patients. The mean ALSFRS-R score changes from baseline showed that the MSC treated group's score decreased at a significantly slower rate for the first four months after treatment, compared to the control group ($p<0.001$) (**Figure 16**) [30]. From four to six months post injection, rate of disease progression increased but the MSC group still showed significantly high ALSFRS scores than the control group ($p=0.003$) (**Figure 16**) [30]. The stability in motor decline seen in the MSC group becomes more significant after the second injection at month 1 (**Figure 16**). However, another study would need conducted that replicated this study but only used one injection to determine if the secondary treatment provided notable benefit in motor performance preservation. Also, it should be discussed that the MSC group appeared to have slightly higher ALSFRS-R scores prior to injection than the control group (**Figure 16**). Whether this is a source of error in this experiment is unknown. Responder analysis added that BM-MSC treated group of patients showed greater functional stability at four months ($p=0.002$) and six months ($p=0.002$) after treatment than the control [30]. These results indicate that the intrathecal injection of BM-MSC cells significantly slowed disease progression and maintain motor function in patients with ALS. This responder analysis data was obtained through observation and therefore could be subject to bias. Unlike the other two clinical trials, levels of various cytokines present in the cerebrospinal fluid were measured in patients before and after treatment. After the first and second injection of BM-MSCs subjects showed a significant decrease in the mean levels of pro-inflammatory cytokines, such as TNF-α and MCP-1, and a significant increase of mean levels of anti-inflammatory cytokines and growth factors, such as TGF-β1–3, IL-6, and IL-10 (**Figure 17**) [30]. It was found, that in subjects who showed the best success with this treatment, there was an inverse relationship between the increase of TGF-β1 and the decrease of MCP-1 at both four months ($p=0.011$) and six months ($p=0.016$) (**Figure 17**) [30]. This correlation was not seen in subjects that response was not as successful. This alleges to MSCs role in reducing reactive glial cells and releasing trophic and anti-inflammatory cytokines to stabilize neuronal environment [21]. These results could be linked to the positive decrease in disease progression identified in this experiment as well. These cytokines' inverse relationship is a steppingstone for identifying a biomarker to detect responsiveness to treatment in future research trials [30]. This trial did provide evidence to the safety and efficiency of intrathecal injections of BM-MSCs as a treatment for ALS, as it decreased the rate of disease progression and reduced amounts of pro-inflammatory cytokines present [30]. Though, this clinical trial did not show prolongment of life in patients, showing that effectiveness of this treatment was only temporary [30]. This study was limited by only screening patients for six months after treatment, which doesn't provide evidence to the long term consequences of therapy [30].

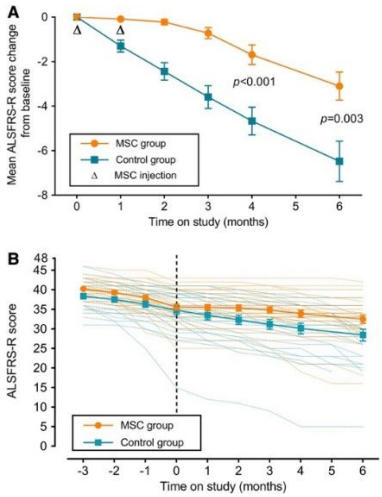

Figure 16: Two graphs that compare the ALSFRS-R scores between ALS patients treated intrathecally with BM-MSCs and the control group. MSC treated patients appeared to exhibit overall less decline in their scores a maintained an overall higher scores in general [30].

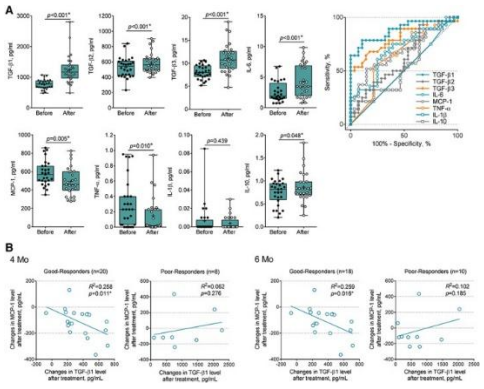

Figure 17: Graphical representation in the changes of amounts of anti- and pro- inflammatory cytokines and growth factors in the cerebral spinal fluid of ALS patients before and after receiving treatment of BM-MSCs intrathecally. There was a decline in pro-inflammatory cytokines and increase in anti-inflammatory cytokines and growth factors. An inverse relationship between the two was only seen in good responders to treatment [30].

All three of these clinical trials proved that intrathecal and intravenous delivery of autologous BM-MSCs to ALS patients is a safe treatment as no side effects were detected. In addition, improvement in the rate of disease was detected by the relative stability of ALSFRS scores [28-30]. However, this stability in motor function appears to only be temporary. Effects on respiratory failure are undetermined as results do not support each other [28, 29]. BM-MSCs appear to prove their functionality and benefits such as minimizing motor neuron damage and producing anti-inflammatory factors. Trials testing only intrathecal delivery found stabilization in disease progression, but the comparative trial between IT and IV delivery found no significant difference between delivery routes [28-30]. Based on evidence that intrathecal delivery allows for more direct spread through the cerebrospinal fluid, IT delivery can consider the more supported route, though direct comparison has not shown clear advantage. Overall, more evidence is needed to prove the most beneficial delivery methods that could possibly prolong survival for these patients.

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Summary & Conclusion

Amyotrophic Lateral Sclerosis is a condition resulting in the deterioration of motor neurons. Categorized as a heterogeneous neurodegenerative disease, the clinical presentation, progressive nature, and causes of ALS are varied making the mechanisms of disease and effective treatments hard to identify. Symptoms of ALS are described as motor dysfunctions, but cognitive and behavioral impairments have also been identified in patients. Disease onset is characterized based on the location in which motor dysfunction begins as either limb-onset or bulbar-onset. Stages of disease are described clinically using the King's staging system or functionally using the Milano-Torino (MITOS) staging system. In clinical trials, the level of functionality is expressed using a scoring system called the ALS Functional Rating System (ALSFRS), which consist of 12 categories of physical function that are each ranked out of 4 points resulting in a score out of 48-points. The ALSFRS is a prime indicator of the quality of life of an ALS patient throughout their course of disease. Though causes of ALS are unknown, studies have suggested that risk factors can be environmental or genetic. Subgroups of ALS can be categorized by heritability into either familial ALS (FALS) or sporadic ALS. Although, both FALS and sporadic ALS patients have been found to contain similar genetic mutations. These genetic mutations have resulted in theories involving cellular mechanisms that participate in the multifactorial pathogenesis of disease. Impaired protein homeostasis, disruptions in RNA processing, morphological changes in glial cells, and excitotoxicity, are just a few of some of the proposed pathophysiological pathways for the development of ALS. In the clinical diagnosis of ALS tests such as nerve conduction studies, electromyograms, and magnetic resonance imaging are conducted to rule out other causes of illness and identify the presence of ALS. There is no cure for ALS and there are very limited treatment options. Riluzole is the only FDA approved medication that has shown success in both slowing the progression of disease and increasing the life span of patients. Because of the proliferation and differentiation abilities of stem cells, research into stem cell therapies for neurodegenerative conditions such as ALS are on the rise. Mesenchymal stem cells are favored in trials as they possess migratory and anti-inflammatory and growth properties, reduce rejection, and neuroprotective capabilities.

Through preclinical and clinical trials, MSCs derived from multiple locations have been paired with various delivery methods to test the effectiveness and safety of stem cell therapies on SOD1G93A rodent and ALS patients. Majority of trials have shown significant success in slowing down the decline of motor functions, without presenting any adverse or dangerous side effects, deeming these therapies safe [28-30]. Specifically, stabilization in ALSFRS scores in ALS patients in clinical trials shows that motor function was maintained for significantly longer. However, these results appeared to only be temporary. The repeated application of treatment did not appear to prolong this stabilization of progression or prolong the life of patients in clinical and preclinical trials. This data also showed that MSC treatment to SOD1G93A rodents has been found to rescue and preserve motor neurons from degeneration in the spinal cord regions, which directly suggests prolongment in motor functions [20, 25]. However, these treatments have not been successful in retaining neuromuscular junctions nor motor neurons present within the muscles. These results give support to the dying back axonopathy theory of ALS [20]. The dying back axonopathy theory is a proposed mechanism of disease progression, which states that before symptom onset distal axon degeneration begins. Degeneration travels in a retrograde direction towards the nerve cell body [3]. In addition, MSC treatment has been found to decrease the presence of over reactive glial cells, which are

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highly connected with the creation of a toxic neuronal environment and neuron death present in ALS [25,26]. MSCs therapies decrease the amount of pro-inflammatory factors associated with this toxic environment, suggesting MSC's phagocytic abilities [25,30]. These results showed that MSCs are also functional in healing the neuronal environment by releasing anti-inflammatory cytokines and trophic factors [25,30]. To better understand these results, it is important to consider the variation among these experiments, such as method of delivery, dose of treatment, single versus repeated administration, and location of cell acquisition. Between the preclinical trials analyzed, the methods of delivery were used: intrathecal, intracerebral ventricular, and intravenous. Only the intrathecal and intravenous delivery methods showed success in temporarily minimizing motor function decline, it is significant to note that these studies utilized MSCs derived from different locations [20,25]. In one clinical trial, intrathecal delivery was compared to the intravenous delivery of autologous BM-MSCs to patients of ALS, and no significant difference in the rate of motor or respiratory decline was found between the two delivery routes [28]. In general, intrathecal delivery has been favored over other methods of delivery. Injection of MSCs directly into the cerebrospinal fluid of the patient allows for the cells to minimize the migration necessary to reach the central nervous system and interact with neurons and the environment [30]. On the other hand, intravenous delivery results in the cells ending up in general circulation increasing the possibility of them getting lost in circulation or stuck in peripheral areas of the body. It is important to record that effects of MSC therapies on respiratory function did not produce consistent data and therefore require further trials to conclude results. Cell dose was another variable factor through the experiments. Doses ranged from 2.5x105 to 4.5x106 (6 uppercase) cells across the reviewed studies, but no clear dose-response relationship emerged when comparing outcomes across trials, likely because dose varied alongside delivery route, cell source, and disease stage at treatment. Further studies controlling for these variables would be needed to determine the optimal cell dose. Throughout both preclinical and clinical trials, the benefits of single versus repeated doses were not evident. The location in which cells were derived can only be compared in the preclinical studies, as all clinical trials utilized bone marrow derived MSCs. Data reveals that hAMSCs and BM-MSCs were both beneficial in decreasing the rate of progression of motor dysfunction, but hUC-MSCs were not. Only treatment with BM-MSC treatment provided evidence of increased survival [20].

In conclusion, stem cell therapies are promising in producing success in treating ALS, through their neuroprotective and anti-inflammatory and growth properties. MSCs treatments have showed to temporarily decrease the rate of progression of motor dysfunction, but no benefits of survivability have been detected. Further research is needed to determine the proper dosage and most beneficial method of deliver and location of cell obtainment.

References

1. Brotman RG (2024). Amyotrophic lateral sclerosis. StatPearls.
2. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, et al. (2017) Amyotrophic lateral sclerosis. Nature Reviews Disease Primers. 3(1).
3. Dadon-Nachum M, Melamed E, Offen D. (2010) The "dying-back" phenomenon of motor neurons in ALS. J Mole Neurosci. 43(3):470-77.
4. Sugaya K, Vaidya M. (2018) Stem cell therapies for neurodegenerative diseases. Adv Experiment Med Biol. 1056:61-84.

5. Bersano E, Sarnelli MF, Solara V, Iazzolino B, Peotta L, et al. (2020) Decline of cognitive and behavioral functions in amyotrophic lateral sclerosis: a longitudinal study. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 21(5-6):373-79.
6. Feldman EL, Goutman SA, Petri S, Mazzini L, Savelieff MG, et al. (2022) Amyotrophic lateral sclerosis. The Lancet. 400(10360):1363-80.
7. Goutman SA, Hardiman O, Al-Chalabi A, Chió A, Savelieff MG, et al. (2022). Recent advances in the diagnosis and prognosis of amyotrophic lateral sclerosis. Lancet Neurol. 21(5):480-93.
8. Mehta P, Raymond J, Nair T, Han M, Punjani R, et al. (2025) Incidence of ALS in all 50 states in the United States, data from the National ALS Registry, 2012–2019. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 26:758-66.
9. Štětkářová I, Ehler E. (2021) Diagnostics of Amyotrophic Lateral Sclerosis: Up to Date. Diagnostics. 11(2):231.
10. Marin B, Boumédiene F, Logroscino G, Couratier P, Babron MC, et al. (2017) Variation in worldwide incidence of amyotrophic lateral sclerosis: a meta-analysis. Inte J Epidemiol. 46(1):57-74.
11. Jiang S, Xu R. (2024) The Current Potential Pathogenesis of Amyotrophic Lateral Sclerosis. Mole Neurobiol. *62*(1):221-32.
12. Zhou W, Xu R. (2023) Current insights in the molecular genetic pathogenesis of amyotrophic lateral sclerosis. Fronti Neurosci. 17:1189470.
13. Young CA, Chaouch A, McDermott CJ, Al-Chalabi A, Chhetri SK, et al. (2024) Improving the measurement properties of the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R): deriving a valid measurement total for the calculation of change. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 25(5-6):400-409.
14. Ramroop H, Cruz R. (2022). Electrodiagnostic Evaluation of Motor Neuron Disease. In *StatPearls*
15. Hoxhaj P, Hastings N, Kachhadia MP, Gupta R, Sindhu U, et al. (2023) Exploring Advancements in the Treatment of Amyotrophic Lateral Sclerosis: A Comprehensive Review of Current Modalities and Future Prospects. Cureus. *15*(9):e45489.
16. Andrews JA, Jackson CE, Heiman-Patterson TD, Bettica P, Brooks BR, et al. (2020) Real-world evidence of riluzole effectiveness in treating amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. *21*(7-8):509-18.
17. McGuigan A, Blair HA. (2025) Tofersen: A Review in Amyotrophic Lateral Sclerosis Associated with SOD1 Mutations. CNS Drugs. *39*(9):903-12.
18. Boopathy K, Palaniyandi T, Ravi M, Wahab MR, Baskar G, et al. (2025) Exploring the potential of stem cell therapy: Applications, types, and future directions. Acta Histochemica. 127(2):152237.
19. Shi M, Chu F, Zhu J. (2025) Stem cells therapy in neurodegenerative and neuroimmune diseases: Current status of treatments and future prospects. Pharmacol Res. 221:107960.
20. Forostyak S, Homola A, Turnovcova K, Svitil P, Jendelova P, et al. (2014) Intrathecal delivery of mesenchymal stromal cells protects the structure of altered perineuronal nets in SOD1 rats and amends the course of ALS. Stem Cells. 32(12):3163-72.
21. Najafi S, Najafi P, Kaffash Farkhad N, Hosseini Torshizi G, Assaran Darban R, et al. (2023) Mesenchymal stem cell therapy in amyotrophic lateral sclerosis (ALS) patients: A comprehensive review of disease information and future perspectives. Iranian J Basic Med Sci. 26(8):872-81.
22. Wyatt TJ, Rossi SL, Siegenthaler MM, Frame J, Robles R, et al. (2011) Human motor neuron progenitor transplantation leads to endogenous neuronal sparing in 3 models of motor neuron loss. Stem Cells Inte. 1-11.
23. López-González R, Kunckles P, Velasco I. (2009) Transient recovery in a rat model of familial amyotrophic lateral sclerosis after transplantation of motor neurons derived from mouse embryonic stem cells. Cell Transpl. 18(10–11):1171-81.
24. Sironi F, Vallarola A, Violatto MB, Talamini L, Freschi M, et al. (2017) Multiple intracerebroventricular injections of human umbilical cord mesenchymal stem cells delay motor neuron loss but not disease progression of SOD1G93A mice. Stem Cell Res. *25*:166-78.
25. Sun H, Hou Z, Yang H, Meng M, Li P, et al. (2014) Multiple systemic transplantations of human amniotic mesenchymal stem cells exert therapeutic effects in an ALS mouse model. Cell and Tissue Res. *357*(3):571-82.

26. You J, Youssef M, Santos J, Lee J, Park J. (2023) Microglia and astrocytes in amyotrophic lateral sclerosis: Disease-associated states, pathological roles, and therapeutic potential. Biology. 12(10):1307.
27. Bosiacki M, Gąssowska-Dobrowolska M, Kojder K, Fabiańska M, Jeżewski D, et al. (2019) Perineuronal nets and their role in synaptic homeostasis. Inte J Mole Sci. 20(17):4108.
28. Nabavi SM, Arab L, Jarooghi N, Bolurieh T, Abbasi F, et al. (2019) Safety, feasibility of intravenous and intrathecal injection of autologous bone marrow derived mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: An open label phase I clinical trial. Cell J. 20(4):592-98.
29. Syková E, Rychmach P, Drahorádová I, Konrádová Š, Růžičková K, et al. (2017) Transplantation of mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: Results of phase I/IIA clinical trial. Cell Transplant. 26(4):647-58.
30. Oh KW, Noh MY, Kwon MS, Kim HY, Oh SI, et al. (2018) Repeated Intrathecal Mesenchymal Stem Cells for Amyotrophic Lateral Sclerosis. Ann Neurol. 84:361-73.