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The Effects of Stem Cells on Amyotrophic Lateral Sclerosis

Willis A and Gallicchio VS*

Department of Biological Sciences, College of Science, Clemson University, USA

***Corresponding author:** Vincent S. Gallicchio, Department of Biological Sciences, College of Science, Clemson University, USA, Email: vsgall@clemson.edu

Research Article

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Abstract

Amyotrophic lateral sclerosis (ALS) is an incurable, cataclysmic motor neuron disease deteriorating the central nervous system. ALS typically has an onset between the ages of fifty to seventy-five with a higher prevalence in white males. The deterioration causes patients to gradually lose voluntary motor abilities to speak, eat, breathe, or move. The main cause of death with ALS is associated with respiratory failure within three to five years of diagnosis. The two pharmaceutical therapies for ALS approved by the FDA are the drugs of Riluzole and Edaravone, however, there has been limited success using this drug therapy. Currently, research using stem cell treatments of Neural Stem Cells (NSC), Human Bone Marrow, Mesenchymal Stem Cells (MSC), Induced Pluripotent Stem Cells (iPSCs), and Human Umbilical Cord Blood Cells (HUCBC) have been projected as an alternative new therapeutic approach. In addition, there are promising results with the addition of lithium to stem cell therapy as a potential therapy for ALS. Stem cell therapy for ALS uses the $SOD1^{G93A}$ transgenic mice model. Human trials using stem cells have been conducted, but additional clinical studies need to be conducted in order to determine the most beneficial stem cell therapy for patients with ALS.

Keywords: Amyotrophic Lateral Sclerosis; Stem Cells; $SOD1^{G93A}$ Transgenic Mice Model; Lithium

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal motor neuron disease deteriorating the central nervous system of the brain, spinal cord, and brain stem [1]. ALS deterioration disturbs the upper motor neurons (UMNs) located in the brain and the lower motor neurons (LMNs) of the spinal cord [2]. Consequently, this degradation causes the patient to lose the ability to speak, eat, breathe, or move as a result of the lost motor connections between the muscles [3]. As the disease progresses, the patient's muscles deteriorate, then eventually lose all voluntary power [3]. The cause of death ALS is most commonly associated with is respiratory failure as a result of the diaphragm muscles becoming incapable of contracting [1]. Most patients are diagnosed with late-onset ALS between the ages of fifty to seventy-five [4]. There is a higher prevalence of ALS in males than females at a ratio of 1.6:1 [5]. There is evidence demonstrating a racial difference recorded by the National ALS Registry in the United States of 2015 demonstrating prevalence in the diagnosis of 100,000 ALS patients comparing whites to blacks at a ratio of 5.4:2.3 [5]. The worldwide incidence rate is approximately two per one hundred thousand [6]. After being diagnosed, life expectancy shows a patient typically has 3-5 years [6].

This disease was first fully discovered and described in the 1880s by Jean-Martin Charcot when he learned motor neurons could degenerate [7]. Charcot graduated from medical school and became a pathologist [8]. Eventually he evaluated approximately 3000 patients with neurological diseases at the hospital that he was working at and separated each neurological disease into

groups [9]. When observing neurological diseases between 1865-1869, he found ALS by noticing that there was a difference of symptoms from damage in the anterior horn and the lateral column of the spinal cord [8]. The anterior damage of the spinal cord results in paralysis with the deterioration of muscles, caused by ALS [8]. There is no cure for the disease as each patient has a different pathology of the affected motor neurons [1]. Studies conducted examined the fundamental roles of motor neuron injury, one being oxidative stress and another being increased levels of glutamate [9,10]. Oxidative stress is the increased volume of reactive oxygen that the body cannot control in order to be able to repair deterioration of the motor neurons [9]. However, there is no specific indication of how ALS forms within the body or risk factors that cause the disease [2]. Ten percent of diagnoses are familial, mostly autosomal dominant, and all other cases (90%) are reported as being sporadic [2]. There are abundant gene mutations presented with ALS and the quality of life is distinctive with every case, leaving the treatment to be unknown and peculiar with every patient (Table 1) [2,11].

Causative Genes	ALS2, ALS3, ALS7, ANG, ANXA11, ATXN2, CFAP410, C9orf72, CHCHD10, CHMP2B, DAO, DCTN1, ELP3, ERBB4, Erlin1, FIG4, FUS, HNRNPA1, LMNB1, MATR3, NEFH, NEK1, OPTN, PFN1, PRPH, SETX, SIGMAR1, SOD1, SPAST, SPG11, SQSTM1, TAF15, TARDBP, TIA1, TUBA4A, UBQLN2, UNC13A, VAPB, VCP
Causative Proteins	TBK1, unc-13 homologue A (encoded by UNC13A), ataxin-2, ubiquilin-2, sequestosome-1, and sterile alpha and TIR motif-containing protein 1, C21orf2, VAPB, transitional endoplasmic reticulum ATPase (encoded by VCP), sec1 family domain-containing protein 1, optineurin (encoded by OPTN), profilin-1, neurofilament heavy polypeptide, tubulin α-4A chain

Table 1: The causative genes and causative proteins associated with ALS [11,12].

SOD1 is the gene mutation second most commonly seen with ALS, specifically 170 missense mutations of the SOD1 gene [13]. Glycogen synthase kinase 3 (GSK-3) is a kinase that plays a role with intracellular communication and is observed in the pathogenesis of ALS because the kinase has signal pathways in the neuron of the cell [14]. If the kinase is overexpressed, this can causes neurodegeneration [14]. In the duration of the disease, GSK-3 has been shown to be elevated, a condition known as hyperphosphorylation [14]. Koh, et al. [14] used the $SOD1^{G93A}$ mouse model to observe the GSK-3 activity in the progression of ALS when using a GSK-3 inhibitor. The inhibitor, {AR-A014418: *N*-(4-Methoxybenzyl)-*N′*-(5-nitro-1,3-thiazol-2- yl)urea}, of the kinase enhanced motor activity of the rodent, postponed the disease onset, and decreased the symptoms during the duration of the disease [14]. Since 1980, there have been more than fifty pharmacologic studies to observe the probable effect of the quality of life in patients with ALS (Table 2) [15]. Most studies have not found a life- changing way to resist the disease [15] (Table 2).

Transfer factor, tilorone, indinavir	Antiviral
Branched-chain amino acids, threonine, lamotrigine, riluzole, gabapentin, nimodipine, dextromethorphan, topiramate, memantine, talampanel, ceftriaxone	Reduces glutamate release, calcium channel blocker, reduces glutamate, NMDA receptor blocker, GABA- analog, glutamate AMPA receptor blocker antagonists, increases astrocytic glutamate transporter activity
Cholinesterase inhibitors, octacosanor, gangliosides, thyrotropine releasing hormone, growth hormone, erythropoietin	Myotrophic effects, systemic trophic factors, ergotropic effects
Ciliary Neurotrophic factor, Insulin- like growth factor 1, BDNF, GDNF, xaliprodene, Granulocyte colony-stimulating factor	Pleiotropic neurotropic receptors, retrograde transport from the muscle axon terminals, serotonin (5HT1A) agonist
Plasma exchange, cyclosporine, total lymphoid irradiation, glatiramer acetate, cerecoxib, minocycline, NP001	Humoral factors, T-cell, microglial suppressor, general anti-inflammatory, vaccination theory, T- helper cells
Acetylcysteine, glutathione, selegiline, vitamin E,	Increases anti-oxidative property, free radical scavenger

CoQ10, edaravone	
Pentoxyfilline, TCH346, minocycline	TNFα linked apoptosis, GAPDH-linked apoptosis
Creatine, acetyl-L-carnitine, dexpramipexole, olesoxime	Mitochondrial membrane permeability stabilizing effects
Phenylbutirate, valproic acid, antisense oligonucleotide treatment	Histone deacetylase inhibitor, blocks production of some proteins
Lithium carbonate, pioglitazone	Facilitates degradation of protein aggregates
Ono-2506	Blocks gliosis
Arimoclomol	Facilitates degradation of protein aggregates

Table 2: Demonstrates the fifty drugs that were tested and the presumed mechanism of each drug in the body [15].

There are pharmacologic treatments using the drugs Riluzole and Edaravone to perpetuate the quality life of patients with ALS, but these drugs only provide moderate effects of slightly longer living compared to the future of stem cell therapy to treat the disease [2]. Within the past few years, advances in new therapeutic strategies have revealed stem cells to be a potential, hopeful method of therapy [2]. Stem cells are thought to be a possible treatment by preventing or repairing the onset of the disease [2]. The pathogenesis of ALS is so sporadic; the therapy of one certain drug is not useful [2]. Therefore, stem cells have the capability to regenerate and restore the impaired neuron cells and their function to succeed as healthy cells [2]. The idea is that one day there will be a successful treatment to ALS [1].

There has been ample amount of research being conducted in animals in order to identify ALS therapy [2]. In mice, the $SOD1^{G93A}$ model has been used in order to demonstrate the effectiveness of stem cell treatments [2]. $SOD1^{G93A}$ transgenic mice are the most common animal model for research of ALS used today [2]. Mice are good subjects because of the size and availability of the subjects to perform like an animal model on Xu L, et al. [16]. The studies using stem cells have shown to effectively replace diseased cells and provide growth from the damage of the disease [6]. Using the model of $SOD1^{G93A}$, a mutation in the superoxide dismutase 1 gene, which is the familial mutation, has proved to have the same pathogenesis in mice as humans with ALS [3]. The downside to these mice is the subjects only demonstrate familial ALS, not sporadic, which is only 10% of ALS cases [15]. This downside transfers over to human trials, because most cases of the disease are sporadic, leaving a question of whether the therapy to be used is actually going to be applicable to humans [15]. The axons of the motor neurons of the mice degenerate the same way as the humans' during the duration of the disease; however, additional axons degenerate in mice than in humans, caused perhaps as a result of the different motor functions when comparing humans to mice [17]. The human onset of the disease can take months or even years, which is significantly greater than rodents with an onset of days [17]. This difference in the time of disease is important to consider because in humans, the stem cells inserted have a different time of maturation and migration in the body [17]. Also, research has been conducted in mice during the pre-symptomatic stage, while in humans therapy is initiated during the symptomatic phase, which can have effects on the onset and pathogenesis of the disease [17].

Research by scientists observing the outcomes of patients with ALS using Neural Stem Cells (NSC), Human Bone Marrow Stem Cells, Mesenchymal Stem Cells (MSC), Induced Pluripotent Stem Cells (iPSCs), and Human Umbilical Cord Blood Cells will be discussed in the review [18-22]. After review of the therapy of stem cells, there are positive advancements towards human stem cell treatment for ALS [2]. Here, it will be discussing current pharmaceutical therapies, multiple stem cell therapies in research using animal models, human stem cell clinical trials, and the projected future for stem cell therapies for human ALS. There will be connections to therapeutic treatments, such as lithium as a potential therapy.

Pharmaceutical Treatments

Since the 1980s, there have been over fifty controlled treatment projects to test potential therapies for ALS (Table 1) [15]. One such project involved using creatine, a compound that is located in the liver, muscles, and the brain [23]. Scientists observed patients with neuromuscular diseases such as Duchenne dystrophy and Becker dystrophy, showed an increase of muscle strength when taking doses of creatine [24-27]. Groeneveld, et al. [28] administered creatine to one hundred and seventy-five patients concluding there was no overall benefit to patients with ALS. Additionally, Shefner, et al. [27] produced the same results as Groeneveld, et al. [28] in a six-month study testing one hundred and four patients of the outcome of administering creatine to conclude no

benefit. Rosenfeld, et al. [23] conducted a nine-month, double-blind study to test the effects of creatine on muscle weakness and quality of life using one hundred and seven ALS diagnosed participants randomly divided to receive a treatment or a placebo. The outcome revealed no muscle strength differences from the placebo group and treatment group [23]. In summary, there has been no strong therapeutic benefit when using creatine in association with ALS [29].

In 1995, the FDA approved Riluzole [15]. Riluzole decreases the supply of glutamate by blocking the positive charge in the channel of the neurotransmitters [30]. Patients with ALS accumulate glutamate causing the death of neurons in the brain [31]. Since glutamate is a central nervous system neurotransmitter, then Riluzole should potentially slow clinical progression while shrinking the amount of the glutamate accumulation [31]. The pharmaceutical approach to use Riluzole has been opposed by professionals due to the only moderate therapeutic results demonstrated through human trials [31]. Bensimon, et al. [32] conducted a double-blind study using one hundred fifty-five patients with ALS with 77 patients taking Riluzole (50 mg twice per day, tablet form) and 78 patients taking placebo pills. The results demonstrated there was a survival rate of fifty-five percent of the treatment placebo group, with minimal side effects of patients having a slight increase of blood pressure, a physical weakening of energy, and more muscle spasicity [32]. Riluzole was approved as a result of two double-blind trials that incorporated 1114 precipitants which demonstrated the death rate decreased by twenty-four percent when compared to the placebo group; however, there was no change in the pathway of the disease [31,32]. Lee, et al. [33] followed the medical charts of 1149 patients living in Taiwan from the years 1999-2008 diagnosed with ALS and using the pharmaceutical treatment of Riluzole to examine if there was a difference in taking the medicine or not. Of these 1149 patients, 451 patients that were administered Riluzole had a mean survival time of an average of 67.75 months after diagnosis compared to the 698 patients with a mean survival time of approximately 50 months [33]. Rooney, et al. [34] conducted a similar analysis of 1,282 Irish patients with ALS from the time period of 1995 to 2010. Observing patients' results, taking Riluzole and visiting the ALS clinic, improved prolonged patient survival to a mean survival time of 17.52 months compared to the patients without taking Riluzole of 10.2 months [34]. The ALS clinic is considered a multidisciplinary clinic because the physicians work directly with a patient with ALS to provide more comfort for problems associated with ALS [35]. These physicians include occupational therapists, social workers, psychologists, neurologists, gastroenterologists, speech therapists, and more specialists to aid the patient [35]. Contradictory, Cetin, et al. [36] tested the effect of Riluzole in ALS patients in Austria from 2008 to 2012 concluding Riluzole only affects survival if taken at the beginning of therapy. The scientists concluded, after six months, the treatment is not beneficial [36]. Overall, Riluzole has only increased the ALS life expectancy of patients by about 3-4 months, without impacting disease progression [2].

In 2017, another pharmaceutical treatment of Edaravone was examined and approved for clinical therapy by humans to prevent oxidative stress when related to ALS [31]. Edaravone is a treatment used for stroke treatment in Japan, showing few detrimental effects [37]. Ito, et al. [37] conducted a trial on $SOD1^{G93A}$ mice using Edaravone and observed a decrease in the disease time progression, demonstrating there is more motor function in treated mice administered Edaravone (Figure 1). The disease progression delayed up to eleven days; when compared to humans, scientists calculated could be equivalent up to twenty months (Figure 1) [37].

In another clinical trial, Yoshino and Kimura increased the amount of Edaravone given to ALS patients from 30 milligrams to 60 milligrams once a day [38]. Not only did the results demonstrate a slower progression of the disease with a higher of dosage of the drug, but when detecting the marker for oxidative stress, there was a decrease of oxidative stress after the drug therapy [38]. Edaravone can be used *in vivo* or *in vitro* and is shown to prevent oxidative stress on neurons to prevent free radicals and reactive oxygen from damaging the neurons [39]. Okada, et al. [40] furthermore conducted a human trial using a treatment group of twenty-seven ALS patients and a placebo group of thirty ALS patients. The treatment group demonstrated an increase in survival rate, with an overall mean survival time of 61 months after diagnosis compared to the placebo group of approximately 32 months.

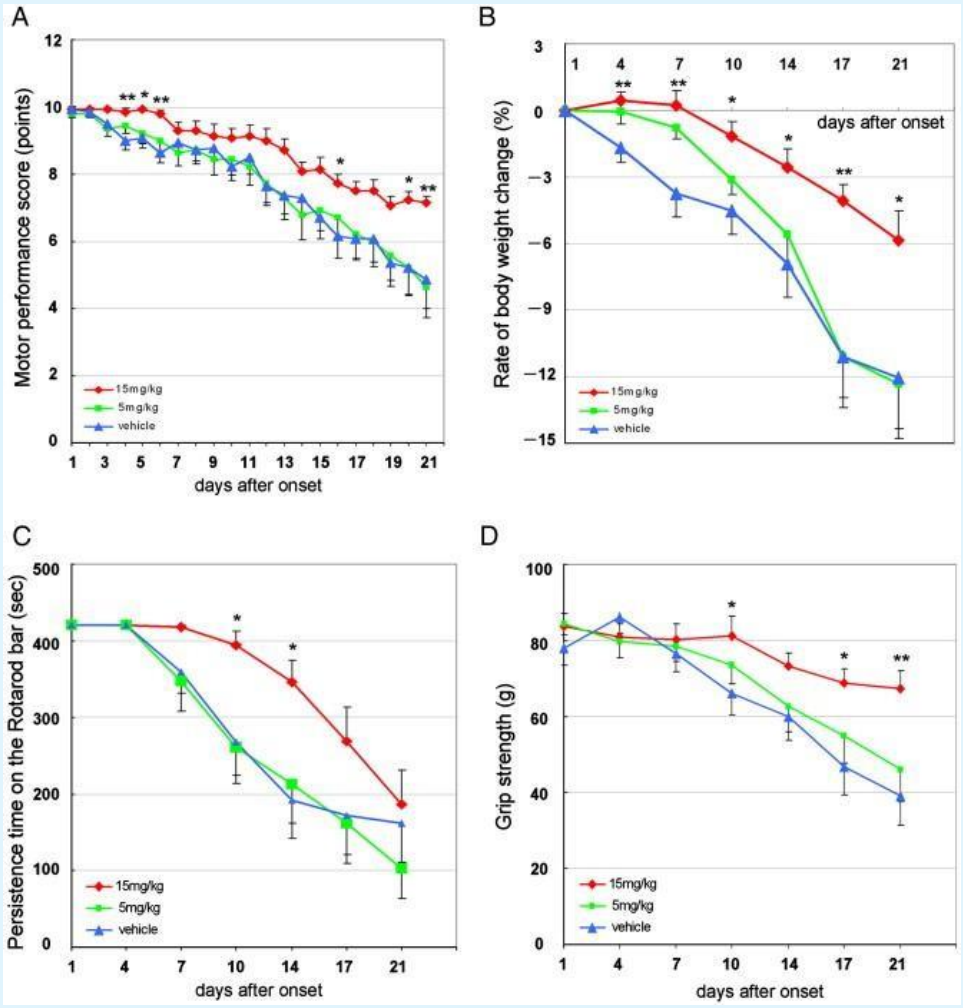


Figure 1: These graphs represent Edaravone treatment in relation to performed tasks. Each graph has a red line to represent a dosage of 15 mg/kg, a green line to represent a dosage of 5 mg/kg, and a blue line of the control vehicle group. **Graph A** compares the motor performance to the days after onset of the disease. **Graph B** demonstrates the rate of body weight change. **Graph C** demonstrates the data found using the Rota-rod test in comparison to the days after onset of the disease. **Graph D** demonstrates the grip strength of the mice compared to days after onset [37].

Neural Stem Cells

Neural stem cells (NSC) come from the neuroectoderm in embryos or adults that leads to the development of any cell in the nervous system [6]. NSCs have the capacity to act as a type of cell needed in the nervous system due to the degeneration of the nervous system [6]. Xu, et al. [20] inserted NSCs from a human fetal spinal cord into a $SOD1^{G93A}$ transgenic model rat [20]. This study found stem cells reformed the broken connection of synapses from neurodegeneration, which in turn postponed the further effects in animals [20]. This led the rats to live 10 days longer than average [20]. Hefferan, et al. [17] performed a study on twenty-four $SOD1^{G93A}$ model rats, 12 females and 12 males, using the injection of NSCs. The study showed that the insertion of NSCs had a larger number of remaining undestroyed neurons, leading to a better lifespan of the animal for functional improvement [17]. Yan *et al*. inserted NSCs from embryonic spinal cords into a 2-month- old $SOD1^{G93A}$ model mouse [19]. The findings demonstrated a longer progression of the neuron disease than comparing to no treatment [19]. Xu, et al. [16]

inserted NSCs into the spinal levels of lumbar and cervical sites of eleven SOD^{93A} model rats. The experimental group received live stem cells, while the control group received the dead graft of NSCs [16]. It was observed that sixty-eight percent of NSCs survived and replaced dead neurons [16]. Rats that had the transplanted stem cells in both spinal levels were shown to have the start of the disease prolonged to 10 days, with an onset around 130 days [16]. The grafted rats lived around 159 days while the control group lived around 142 days [16]. The experimental group lived 17 days longer than rats of the control group [16]. Lee, et al. [21] inserted NSCs into the spinal cord for forty $SOD1^{G92A}$ mice and found the symptoms delayed for seven days and their lifespan was extended by twenty days. With this knowledge, Teng, et al. [41] accessed and reviewed eleven studies on the use of NSCs that have been proved to delay the onset time for the development of the disease, increase the time for the disease progression post-diagnosis, and extend the lifespan of the animal (Figures 2 & 3).

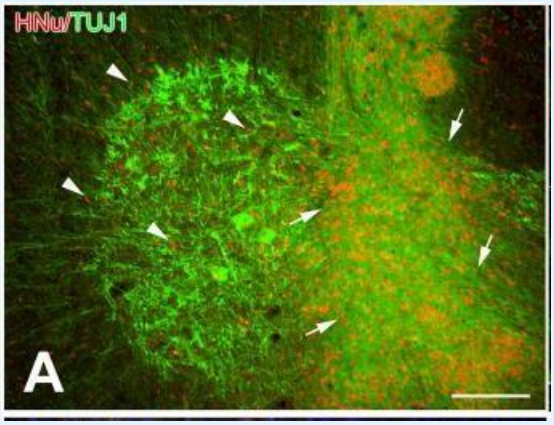


Figure 2: This study conducted by Xu, et al. [16] shows the progression of the grafted NSCs in the mice. This picture demonstrates, the live grafted NSCs migrating to the cervical cord (colored red). The white arrows represent the sites of inoculation.

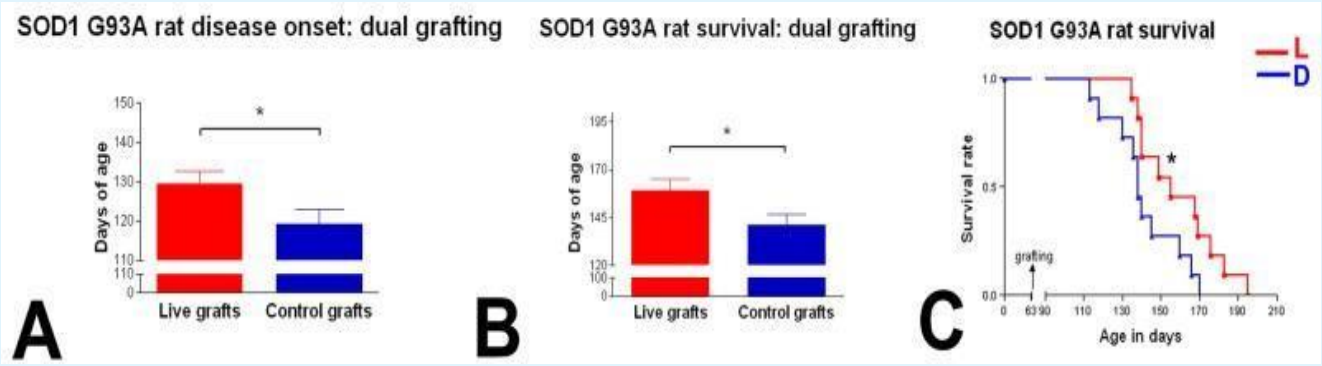


Figure 3: This study conducted by Xu, et al. [16] shows the progression of the grafted NSCs in the mice.

A. Graph A demonstrates the disease onset to the grafting of the NSCs. It is shown that the disease onset for live grafts were approximately 129 days compared to the control grafts of approximately 119 days.

B. Graph B compares the survival rates of the injection of NSCs. It is shown that with the injection of the live grafts, the rats lived approximately 158 days compared to the control grafts of approximately 141 days.

C. Graph C is comparing the course of the disease throughout around 180 days. It can be observed that the rats with the injected live NSCs have a higher survival rate.

As shown using human neural cells Xu, et al. [16], Lee, et al [20], Xu, et al. [21] found the use of stem cells prolonged the onset of the effects of the disease. In July of 2011, the first phase of a clinical trial on humans was performed, and in May of 2013, the second phase of the clinical trial was performed [42,43]. In 2011, in the U.S. phase I trial, 100,000 fetal spinal cord NSCs were injected five times into the spinal cord of six ALS patients, two females and four males [42]. The patients were all confirmed with sporadic ALS [42]. Some of the requirements included the patients being between the ages of twenty and seventy-five, evident progression of the disease, no current pregnancy, no psychiatric disease, etc [42]. NSCs were extracted from a fetal *in utero* death

spinal cord [42]. After surgery, neuroimaging, blood tests, and a clinical psychologist observed the patients [42]. After reviewing the patients six to eighteen months post-injection, there was no disease progression on motor movement [42]. Patients span the ages of 30, 35, 38, 54 57, and 67 [42]. The older patients of 57 and 54 died within eight months post-surgery due to a respiratory failure [42]. Sixty-seven of the patients passed away, but the cause is not specifically evident, due to the family not wanting to release information [42]. Scientists confidently think that this injection of NSCs is a safe procedure [42]. Phase II aimed to test fifteen patients with the safety of doses of increasing number of cells per injection and the number of injections [45]. Two patients that had received 20 injections with 400,000 cells per injection showed detrimental effects of complications with one having spinal cord swelling and another having severe pain, but four more patients with that dosage had no complications [43]. The other patients received 20 injections at various concentrations but had no complications from the injections [43]. Scientists concluded, with careful watch, a patient can tolerate the high injections of 20 with a concentration of 400,000 stem cells [43].

Human Bone Marrow

Human bone marrow stem cells have been tested in $SOD1^{G93A}$ mice as a potential therapy for ALS [44,45]. Stem cells present in human bone marrow are hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) [44]. The therapeutic rationale is that stem cells are going to regenerate the deteriorated tissue specific cells and also protect the cells from further damage [46]. Garbuzova- Davis, et al. [22] studied the effects of repairing the blood-spinal cord barrier in ALS by injecting human bone marrow stem cells ($CD34^{+}$) in $SOD1^{G93A}$ mice using three increasing cell doses ($5x\ 10^4$, $5x\ 10^5$, and $1x\ 10^6$ cells) at thirteen weeks of age [22]. Comparing the progression of ALS between mice and humans, there is a direct correlation with capillary endothelium damage, which is the damage of cells lining the vascular system [22]. Scientists observed with high dosage human bone marrow stem cells increased the delay disease onset and maintained the survival of motor neurons [22]. Human bone marrow stem cells replaced the endothelial cells to stop the damaging progression of the capillaries of the spinal cord [22]. Eve, et al. [45] continued the findings reported by Garbuzova- Davis, et al. [22] by conducting a similar study inserting the same doses of $5x10^4$, 5×10^5, and 1×10^6 of human bone marrow stem cells to observe the blood-spinal cord barrier during the progression of ALS. There were significantly less microhemorrhages in the cervical and lumbar of the spinal cord of the treatment group injecting high dosages ($1x10^6$) when compared to medium and low doses [47]. Gubert, et al. [48] injected in vivo bone-marrow mononuclear cells from an adult femur and tibia into the spinal cord before the ALS symptoms were present, at 9 weeks, and then after the symptoms were present at 14 weeks. Rota-rod, hanging wire and the motor score tests were used as a detection method of the motor functioning comparing the two groups [48]. The Rota-rod test is used when the mouse is placed on a rotating rod to record how long the mouse stays on without falling off [48]. The hanging wire test is an examination when an animal is placed on their cage recording the time when they fell; specifically, this test indicates the limbs strength [48]. The motor test score specifically looks for motor dysfunction and paralysis of limbs [48]. The motor score test ranks animals 0-4 based on the motor function of the limbs: 4= no sign of motor deterioration, 3=slight motor dysfunction and tremors in limbs, 2= obvious motor dysfunction, 1= completely no function of a limb, 0= almost complete loss of function of limbs, unable to pick the body up with legs when placed not upright on feet [48]. (Figure 4) demonstrates the results of the motor tests completed compared to the age of pre-symptomatic, saline injected, current symptomatic, bone marrow mononuclear cells, and the wild-type ALS mice without treatment [48]. In all tests, the treated stem cell groups showed longer/ better scores in each category, especially in the pre-symptomatic phase [48]. There was not as much increase as the scientists hoped for in the symptomatic stage, which needs to be observed [48]. In this study, there was a discrepancy in the results- one treatment group had a prolonged lifespan while another one did not; however, there was an increase in the motor skills of the mice [48]. When comparing the males and females, females have a longer lifespan than males [48]. This longer lifespan is predicted to be from estrogen, a protective female hormone, but the numbers of males and females equal out at a decline of age [49]. Another discrepancy arises because humans are only treated in the symptomatic phase, while in the study, mice are treated in the pre-symptomatic phase are compared to mice treated in the symptomatic phase [48].

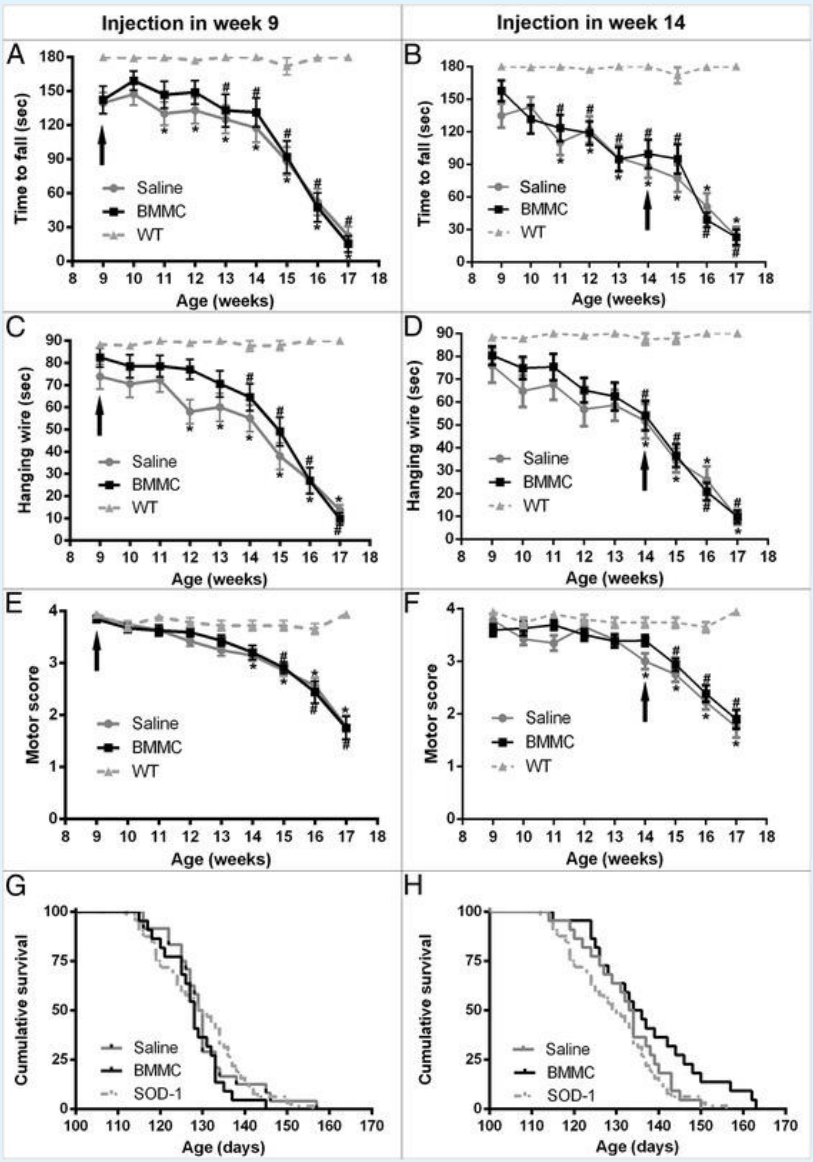


Figure 4: **Graph A** represents the time to fall off compared to the age of the mice with the injection at 9 weeks. **Graph B** represents the time to fall compared to the age of the mice with the injection at 14 weeks. **Graph C** represents the time to fall off the hanging wire of rats compared to the age of the mice with the injection at 9 weeks. **Graph D** represents the time to fall off the hanging wire of rats compared to the age of the mice with the injection at 14 weeks. **Graph E** represents the motor of rats compared to the age of the mice with the injection at 9 weeks. **Graph F** represents the motor of rats compared to the age of the mice with the injection at 14 weeks. **Graph G** represents the survival compared to the age of the mice with the injection at 9 weeks. **Graph H** represents the survival compared to the age of the mice with the injection at 9 weeks [48].

Rando, et al. [44] studied the effects of bone marrow by inserting bone marrow stem cells into the quadriceps muscles of mice to observe an increase in the functioning motor skills and the decrease the motoneuron degeneration. There was no significant delay in the onset of the disease, but the progression was slower in the bone marrow transplanted group [44]. Scientists used the Rotarod test to observe the physical capabilities of the mice

throughout the progression of the disease comparing the bone marrow injected and the culture media control group, which the treatment group showed an improvement of the rod test compared to the control group [46]. The treated group survived approximately six days longer than the control group [44]. Terashima, et al. [51] conducted a study using human bone marrow with an enhanced stem cell factor in thirty-four $SOD1^{G93A}$ mice. There were three groups receiving therapy of bone marrow, stem cell enhanced bone marrow, and FMS-like tyrosine kinase 3 (flt3) bone marrow cells [51]. Scientists observed mice using the Rota-rod test, which is a rod that the animal is placed on while rotating at whatever speed the scientists choose, so the animal must continuously walk to not fall off [52]. The Rota-rod test was used to observe the physical capability of the mice in the progression of the disease while comparing the activated cells with the non-activated [52]. The study found that the activated bone marrow cells showed an increase in survival and function of the animals compared to human bone marrow (Figure 5) [51]. One can observe the wild-type $SOD1^{G93A}$ mice with activated stem cell factor bone marrow stem cells completed longer seconds of the Rota-Rod test and survived the longest around 23 weeks (Figure 5-B, C) [51]. Scientists also observed the spinal cord of these animals by observing the progression of the activated stem cells in the spinal cord [51]. This study concluded not only does bone marrow have a superior effect on the progression of ALS in transgenic mice, but the stem cell bone marrow can enhance these effects greatly [51].

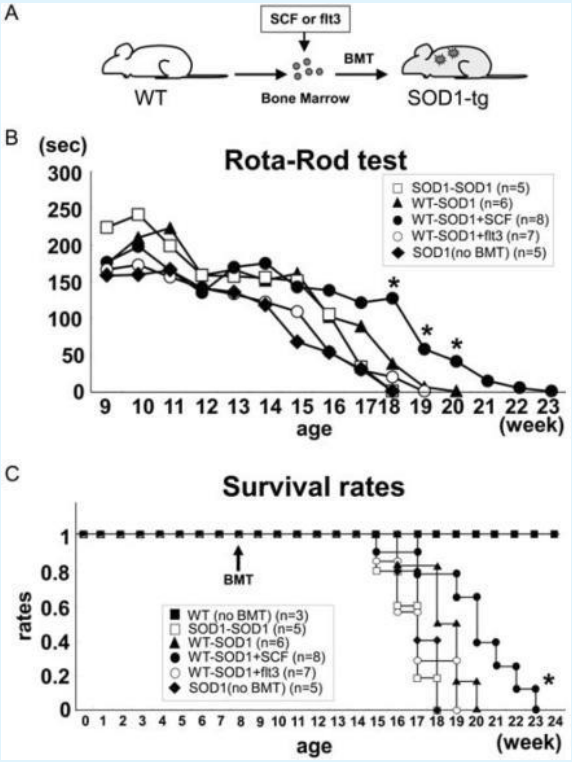


Figure 5: Photo A, the transition of wild-type (WT) mouse into a SOD1 transgenic mouse (SOD1-tg) using the activated stem cell factor human bone marrow or the tyrosine kinase 3. Using the Rota-Rod Test, **graph B**: shows the rotation of the rod (in seconds) compared to the age of the SOD1 ^{G93A} mice with ALS ranging from 9 weeks to 23 weeks of survival. There are five groups represented of SDO1 mice that were treated with bone marrow from SOD1 mice (SOD1-SOD1, open squares), SOD1 mice with no treatment (WT-SOD1, black triangles), SOD1 mice treated with human activated bone marrow (WT-SOD1+SCF, black circles), and SOD1 mice treated with tyrosine kinase 3 (WT-SOD1+flt3, open circles), and SOD1 transgenic mice (SOD1 no BMT, black diamond). **Graph C** demonstrates the survival rates of the five groups of treatment [50].

Mesenchymal Stem Cells (MSC)

Mesenchymal stem cells are taken and isolated from human bone marrow, so this can limit the safety concerns of the transplantation [52]. The reason scientists began to use these cells is that it has been observed that when injected into a spinal cord that is demyelinated, the cells regenerated to create myelination [53]. This remyelination even proved to create a better electricity connection in the spinal cord than before, demonstrating its potential [53]. These stem cells are reasonable to have contained and transport to whenever is needed to go [54].

Vercelli, et al. [52] injected mesenchymal stem cells, 800,000 stem cells, into the lumbar spinal cord of $SOD1^{G93A}$ transgenic mice. Scientists observed the mice by performing motor tests, Rota-rod tests, and wire hanging tests [52]. The treatment group had longer lasting effects of the disease compared to the control group, showing this that there was a decrease of motoneurons proving why the physical activity was higher in the treatment group [52]. There is an interesting review that males are quicker at demonstrating a progression of the disease than females, the explanation is still unknown [52].

Oh, et al. [55] wanted to test the safety of injecting intrathecal, which is in the spinal cord that can directly affect the spinal fluid, mesenchymal stem cells into eight patients (approximately $15x10^6$ in each patient). The study concluded there were no harsh effects outside of slight pain and a headache. Syková et al. [56] conducted an eighteen-month study using twenty-six patients with a lumbar injection of mesenchymal stem cells [56]. Just as previous studies have down, the precipitants had mild effects of the drug of headaches, but no serious complications.

Karussis, et al. [57] conducted a clinical trial on fifteen patients with multiple sclerosis and nineteen patients with ALS, injecting mesenchymal stem cells to observe the outcome. Some patients had mild side-effects with twenty-one patients observed to have an immediate fever, fifteen patients reported having a headache, and two patients with leg pain proving no detrimental effects for the follow-up of six to twenty-five months demonstrating the safety of administering the stem cells [57]. Scientists also observed patients MRI scan up to one year after treatment, showing the condition of the patient did not change [57]. Bonab, et al. [58] observed the effects of administering mesenchymal stem cells into ten patients with multiple sclerosis, showing there were no adverse effects, but there were no promising side effects in symptoms in patients only showing two patients with little positive results [58]. Mazzini et al. [59] first demonstrated using mesenchymal stem cells transplanted into the spinal cord of humans is safe because no patients suffered harsh effects, except reversible pain in legs. Then, Mazzini, et al. [60] was the first surgical study on patients with ALS using the transplantation of mesenchymal stem cells. Scientists evaluated ten patients with sporadic ALS to inject a variety number of MSC to observe the patients every three months by performing muscle tests given by a neurologist and physiotherapist, also observing the patients MRI [60]. Scientists found there were no adverse effects from the drug with only a few days of pain and more than half showed sensitivity in a leg, concluding this use of mesenchymal stem cells are a potential therapy for ALS [60]. In addition, there was a slight increase in lifespan of 5 patients [60]. There is still a ways to go in research because the sites of inserting are still are question and the number of cells at the time of injection for the largest benefit [60].

Lithium

Lithium may serve as a potential therapy for patients with ALS, specifically discussing therapy using $SOD1^{G93A}$ models and clinical trials. There have been human clinical trials conducted on ALS patients using bone marrow stem cell transplants. In research, the effective recommended dosage of lithium is 900 mg per day and the plasma lithium levels should be above 0.5 mEq/L [61]. Sharma, et al. [46] conducted a retrospective study using thirty-seven patients in the treatment group that received an intrathecal bone marrow stem cell transplant, around $8.5x10^6$. A twenty person control group received no treatment [46]. All patients were diagnosed with ALS in the treatment group or placebo group observing changes in the survival rate [46]. Each group was divided into subgroups based on age below and above 50, then divided depending on the onset of the disease (bulbar or limb) [46]. Groups were further divided to receive lithium or to not receive lithium [46]. Each patient was prescribed 300 mg of lithium twice a day for six weeks orally [46]. Fifteen patients in the treatment group were prescribed lithium and twenty-two patients of the treatment group were not prescribed lithium [46]. The ALS control group not prescribed lithium lived approximately 66 days [46]. Patients who were diagnosed below the age of 50 lived approximately 113 days while above the age of 50 lived approximately 63 days [46]. Patients receiving lithium in addition to the bone marrow cell transplantation demonstrated a higher survival rate of around 106 days

[46]. The age of patients was considered, demonstrating the younger the age, the higher the survival rate [46]. There is a downfall to this experiment only having a thirty-seven patient treatment group, and then further divided into many different treatment groups, making the conclusions not as strong [46].

There have been any additional studies using with lithium therapy on patients with ALS. Lithium carbonate (Li2CO3) is used in psychiatry of the treatment of choice for suicide, depression, and bipolar episodes [62]. Turner, et al. [63] observed the connection between ALS and psychiatric disorders such as schizophrenia, bipolar disorder, depression and anxiety especially within the first year of diagnosis [63]. Longinetti, et al. [64] also found a link in psychiatric disorders and ALS with a 49% increased risk of developing ALS if a psychiatric disorder was present. Also, if the patient with ALS has children, the children have an increased risk of developing a psychiatric disorder [64]. In patients using lithium for psychiatric treatment, the plasma lithium levels between 0.4- 1.2 mEq/L is generally effective [65]. Lithium therapy has been considered because it has been shown to block the production of mutated genes, reduce oxidative stress in cells, and promotes the generation of healthy cells in the brain [62]. Fornai, et al. [66] conducted a mEq/kg) and twenty $SOD1^{G93A}$ mice with the placebo of saline. Scientists concluded lithium bicarbonate increased the number of days of the onset of limbs adduction, the onset of paralysis, and the number of survival days with treatment compared to the placebo (Table 2) [66].

	The onset of limbs adduction (number of days)	The onset of paralysis (number of days)	Survival Days (number of days)
SOD1 Mice	approx. 101days	approx. 109.8 days	approx. 110.8 days
SOD1Mice+ Lithium Bicarbonate	approx. 109.2days	approx. 145.1 days	approx. 148 days

Table 2: A chart categorizing the onset of limbs adduction, the onset of paralysis, and the length of survival for transgenic $SOD1^{G93A}$ mice treated with saline and $SOD1^{G93A}$ mice with a daily intake of lithium bicarbonate [66].

With promising results on $SOD1^{G93A}$ transgenic mice, Fornai et al. [66] translated his studyinto a 15-month clinical trial on human patients to observe the findings for lithium carbonate therapy. Sixteen patients (8 female, 8 male) diagnosed with sporadic ALS were given lithium carbonate therapy of (150 mg doses/ twice a day) plus one tablet of Riluzole (50 mg/ twice a day). The twenty-six person (16 female, 12 male) control group only received tablets of Riluzole (50 mg/ twice a day) [66]. During the study trial when lithium plasma levels of were below 0.4 mEq/liter, lithium dosage was raised from 300 mg per day to 450 mg per day to ensure the lithium plasma levels were between 0.4-0.8 mEq/liter, but the study did not include the amount of time needed to take the higher dosage to reach the effective lithium plasma levels [66]. Results concluded 100% of patients were alive in the lithium treatment group while only 81% of patients were alive in the control group; demonstrating lithium decreased the progression disease time [66]. Scientists showed positive results in transgenic mice and in patients testing their hypothesis that lithium by demonstrating in transgenic mice there were a larger amount of neruons [66]. The lithium treatment transgenic mice demonstrated a longer lifespan of approximately 148 days and the non-treatment group had a life span of approximately 110 days [66].

Aggarwal, et al. [68], UKMND-LiCALS study group, et al. [69], Miller, et al. [70], Boll, et al. [71] conducted studies to observe the effects of lithium intake on ALS disease progression (Table 3).

Scientists	Type of study	Number of Patients	Control Group	Treatment Group	Lithium Plasma Levels	Disease Progression
Aggarwal *et al.* (2010)	Double- blind placebo controlled	84	Riluzole (44 patients)	Lithium Carbonate 150 mgcapsules(3 perday) +Riluzole(40 patients)	0.4-0.8mEq/L,if levelbelow0.4 mEq/L then an additional tablet was taken	No significant decrease of disease progression
Miller*et al.* (2011)	13-month controls	356	Placebo Tablet (249	Lithium Carbonate 150 mg (twiceaday) and	0.3–0.8 mEq/L,if levelbelow0.3	Concluding lithiumisa poor candidate

			patients)	sometimes increasing/decreasing (107 patients)	mEq/L, then raised dosageto 450mg/daymax	for testing
UKMND-LiCALS study group *et al.* (2013)	Randomized, Double-Blind, Placebo-Controlled Trial(18 months)	214	Placebo Tablets (107 Patients)	Lithium Carbonate 295 mg daily(107 patients)	0.4-0.8 mEq/L	Nosurvival difference between the groups, but nosafety concerns
Boll *et al.* (2014)	21-month study	49	ALS patients, no treatment (31 patients)- - 5 of them treated with Riluzole	LithiumCarbonate (150 mgto600mg duringfirst 15days) thendoses adjusted to(0.3-0.75 mEg/l) and Valproic Acid (200 mg) (18 patients)	0.3- 0.75 mEq/L	Increased survival rate (p=0.016), oxidative defense increased, but trial stopped due to adverse effects from patients

Table 3: Four groups of scientists conducted studies on patients with ALS comparing treatments of lithium carbonate and placebo groups. Each group of scientists did not find any significant decrease in disease progression [68-71].

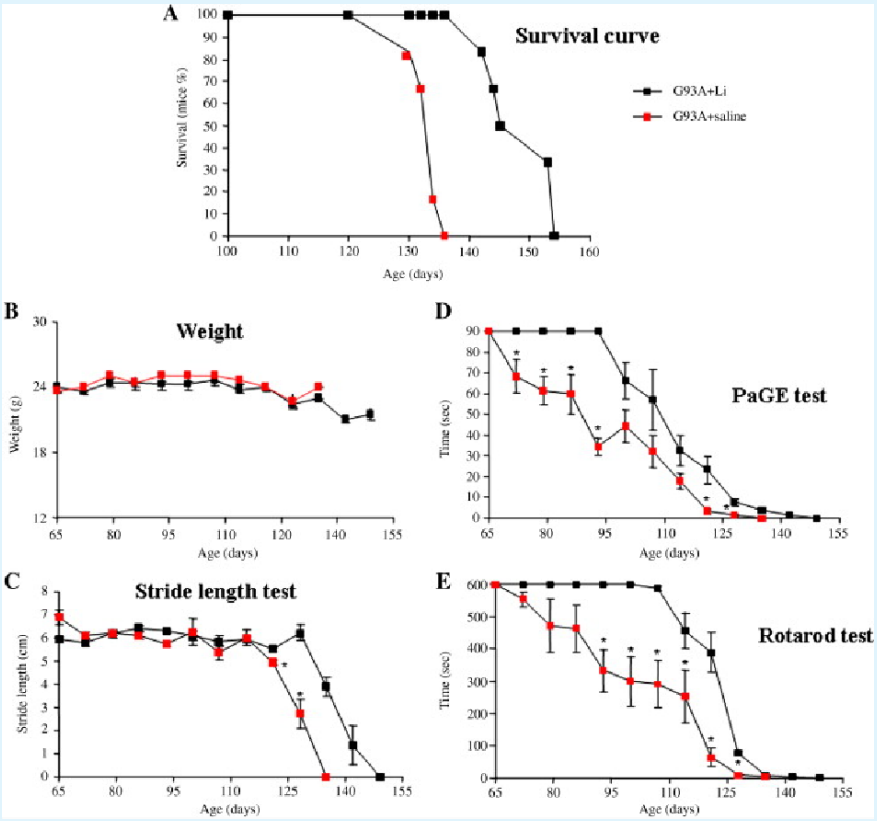
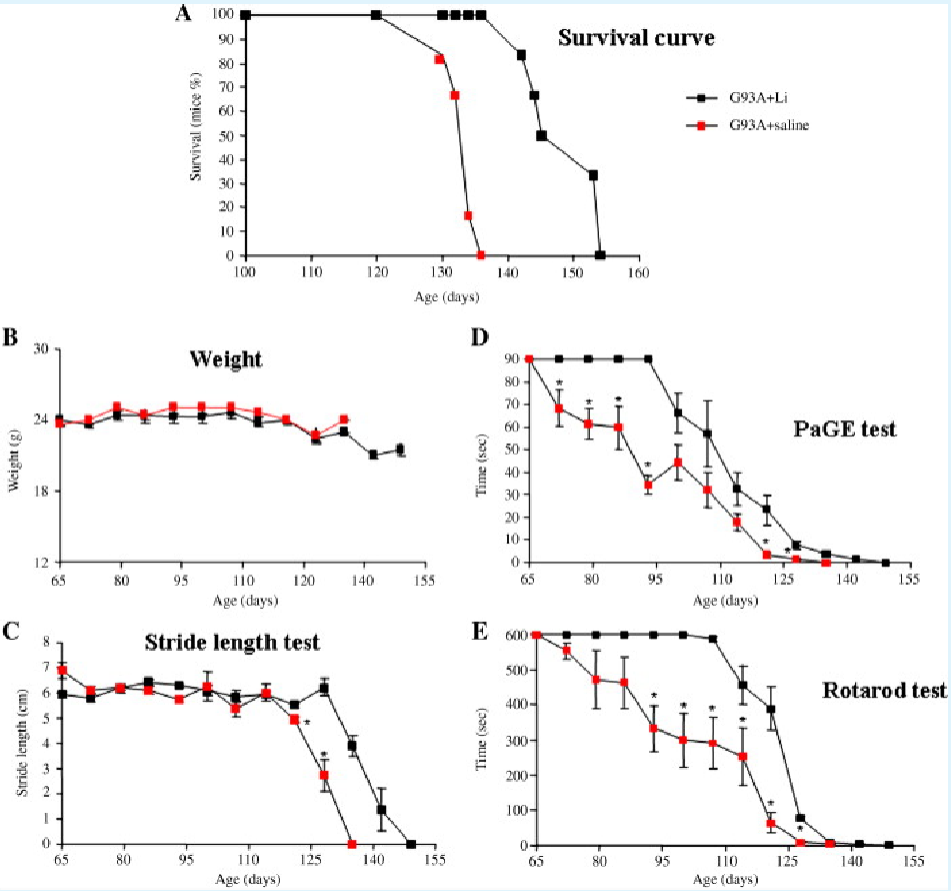


Figure 6: The legend demonstrates the black line as the transgenic $SOD1^{G93A}$ mouse with the treatment of lithium chloride and the red line as the transgenic $SOD1^{G93A}$ mouse with saline solution treatment. **Graph A** represents the overall survival curve on an average of the control group surviving approximately 138 days and the treatment group surviving approximately 153 days. **Graph B** represents the weight changes in the two groups, which was not a big interval change in the weight. **Graph C** represents the data from the stride length test, demonstrating higher values in the treatment group**. Graph D** represents the paw grip endurance test showing all higher results than the control group. Graph E represents the Rotarod test demonstrating greater balance and strength of mice with the treatment of lithium [41].

When observing (Table 3), each experimental test was conducted with less than the recommended daily dose, 900 mg per day, and the lithium plasma levels should have been required to be at least at the threshold of 0.5 mEq/L.63

Fornai et al. [72] studied transgenic sixty-seven day old SOD1G93A mice split into the groups: Five SOD1G93A lithium chloride (1mEq/kg) treated every other day, five SOD1G93A wild-type lithium chloride (1mEq/kg) treated every other day, five SOD1G93A saline-treated, and five SOD1G93A wild-type saline-treated. Scientists collected information (Figure 6) by the stride length test: an open area in a box with a lit area and dark area and while the mouse ran around the stride length was taken between the two paw prints, the paw grip endurance test: a mouse placed on a wire and the wire was shaken for 90seconds to observe the time on the grid, rotarod test: mouse placed on a rotating rod (15 rpm) and the time observed stayed on (10 minutes maximum) [72]. (Figure 7) demonstrates the findings of the scientists in line graphs. Overall, the treated group SOD1G93A transgenic mice group treated with lithium chloride had an increased overall result in the number of days in comparison to the control group of SOD1G93A treated with saline [72].



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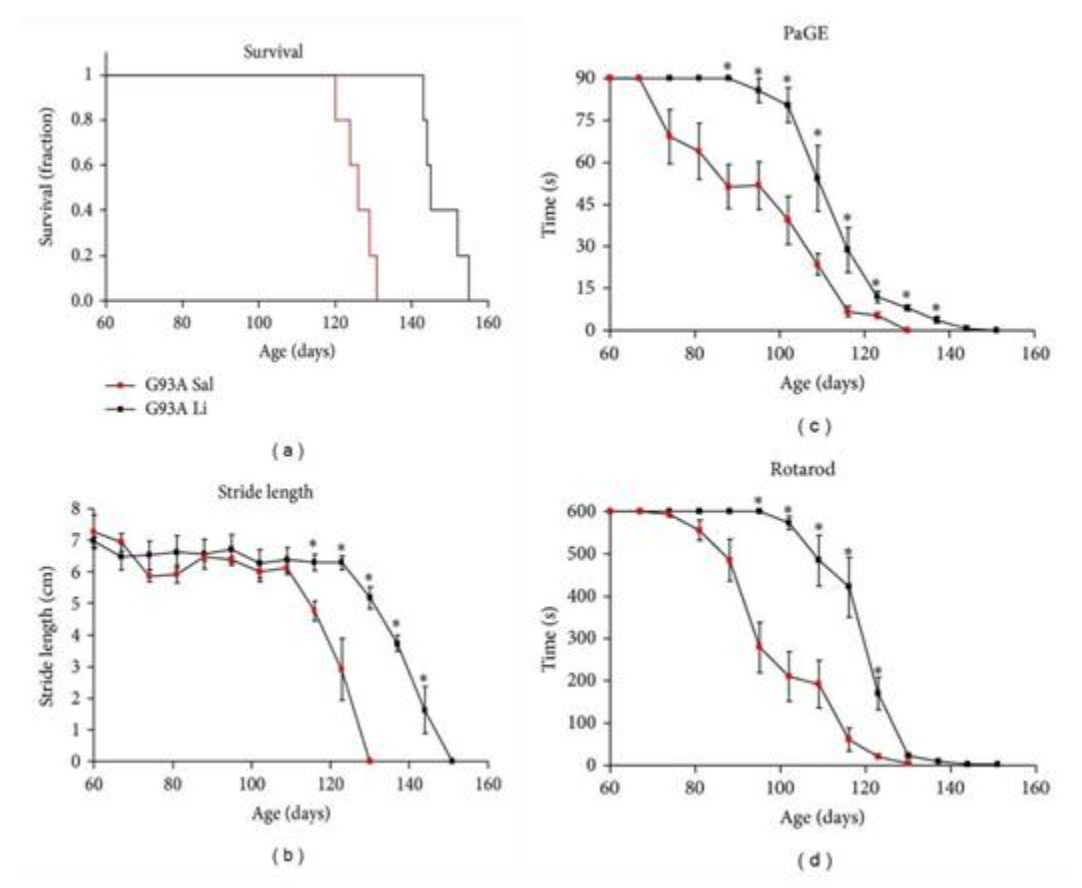

Figure 7: Graph A represents the survival fraction compared to the age (in number of days). Graph B represents the stride length (cm) vs. the age (in number of days). Graph C represents the paw grip endurance test in time (seconds) vs. age (number of days). Graph D represents the Rotarod test with time (seconds) vs. age (days). Throughout the graphs, the right lines or dots.

Represent SOD1G93A mice treated with saline. The black lines or dots represent SOD1G93A mice treated with lithium chloride [72].

Scientists concluded lithium protects against the neurodegenerative breakdown from the disease [72]. Scientists also demonstrated the addition of lithium was protective and neuronal markers in the spinal cord with active stem cells demonstrating plasticity and neuronal survivial [72].

Natale, et al. [73] described beneficial effects of lithium.75 Scientists divided the study into four groups using used five SODG93A transgenic mice treated with lithium chloride (1 mEq/Kg), five wild-type mice treated with lithium chloride (1 mEq/Kg), five SODG93A with vehicle saline sodium chloride, and five vehicle saline sodium chloride treated wild-type mice. All of the mice were treated in vivo in the pre-symptomatic stage of the disease [73]. Scientists observed the changes of the mitochondria such as the changes in the dendrites, density, and motor axons [73]. Scientists also inserted the lithium in vitro from the ventral spinal cord of the mice [73]. Lithium chloride showed remarkable muscle degeneration protection when viewed under a microscope in the muscle cells [73]. As discussed in the introduction, glycogen synthase kinase 3 (GSK-3) is a causative factor in the pathogenesis of ALS.76 Lithium targets to block GSK-3 [74]. It has been shown with dosages of lithium such as 2 mM, significantly phosphorylated GSK-3, but with lower dosages like 0.5 mM made no changes to the kinase [75]. By phosphorylating GSK-3, genes are enhanced for growth, neuroprotection, and also able to differentiate [74]. These

results demonstrate why lithium could be used as a potential therapy to hinder the pathogenesis of GSK-3, which causes more gene transcription of healthy cells [62]. They conclude the plasma lithium levels in patients is important with 0.5 mM as the threshold is greater than 0.5 mM induces GSK-3 inhibition, while lithium values below 0.5 mM do not corelate with clinical response. Induced Pluripotent Stem Cells (iPSC) Induced pluripotent stem cells (iPSC) are derived from skin or blood from adult cells that have been transformed back into an embryonic-like state that enables the causes the cells to be 30 used wherever needed.78 This iPSC technique is a safe method because it comes directly from the patient itself, leaving the possibility of rejection or infection limited [76]. There are limited ethical 77concerns with iPSCs because the cells come from the patient's own cells [76]. The cells derived from the skin or blood is grown in a dish, to in-turn multiple by the millions, leaving an abundant of cells to have right at the fingertips [77,78]. Using this technology, the pathway of ALS can now be more monitored to learn the pathogenesis as the disease progresses [77]. However, the cost of using this technology is more than thousands of dollars and even more depending on the type of cell needed to derive, causing many people to not participate due to cost.81 Su et al. demonstrated the high yield of cells derived from mesenchymal cells, 40,000 stem cells, using iPSC technology in vivo to then be injected into mice [77]. The data showed there were new connections of axons in the mouse forming action potentials in the muscles to decrease muscle atrophy [76]. This technology now demonstrates the technology used from deriving neural stem cells and human embryonic stem cells [80].

Cord Blood

Cord blood is known to contain hematopoietic stem cells that originate from a baby's umbilical cord and can be extracted to be used for the same uses when compared to blood or bone marrow [82]. Cord blood has clinical uses are for just about anything because of the differentiability including tissues, and many different cells [82]. Although parents can make the choice to store the cord blood from the baby's umbilical cord after giving birth, there are no current testing on the diseases that could be present from storage or confirmed the best storage for the blood [22]. Garbuzova-Davis, et al. [82] first demonstrated the migration of the cord blood cells (a dosage of 1x106 cells) to the neuron affected places, which demonstrated restoration and 31 preservation of the damaged neurons by also observing the delaying of the symptoms of the disease. Next, Garbuzova-Davis et al. demonstrated at different doses of human umbilical cord blood and observed the results for many weeks [81]. The researchers exhibited the medium dosage of cells (25x106 cells) had the longest survival rate of approximately twenty-six weeks of age, compared to50x106 cells had a lifespan of twenty-two weeks of age, but longer than the control group of twenty-one weeks of age [81]. On top of the postponed advancement of the disease and the prolonged life expectancy, the middle dosage of cord blood cells also showed a significant effect in aspects of inflammation, heightened number of lymphocytes (the form of small white blood cell) [81]. These two studies have shown to increase the knowledge of the effects of the injection of cord blood cells and thecorrect dosage of cells [81,82]. Knippenberg, et al. [83] conducted a supportive study to evaluate the effects of intraspinal injecting human embryonic cord blood on two groups of transgenic SODG93A mice models of ALS before onset and after onset. The before symptom group (day 40) and the symptomatic group (day 90), during injection there was no signs of impairment of the mice 83 injection except one complication [83]. Scientists observed and concluded the early the dose of the cord blood, the expanded time of endurance, less neuron loss, and improvement of achievement from the body's movement with concluding the day 40 group had a better outcome of results than the day 90 group, also with females having to increase significant results.85 The study concluded more testing should be done predicting different times of injection, different places of injection, and doses with symptoms to conclude the best dosage of cord blood on mice before human trials [83].

Conclusion

More research needs to be completed studying ALS; however, there has been much progress since the discovery of this fatal disease. Since all of the research is mainly completed on the SOD1G93A transgenic mouse model which only can account for 10% of ALS cases, there needs to more clinical trials for stem cell studies [84]. There is speculation that the pathogenesis of the disease could be different for sporadic and familial, leaving using the SOD1 model somewhat unreliable [84]. Many studies of research start the mice on stem cell therapy during the presymptomatic stage of the disease and this can cause discrepancies because clinicals trials start stem cell therapy after the onset of the disease [84]. Since each patient requires a different treatment, will scientists ever find a correct treatment for therapy? (Table 4) represents an overall conclusion with different stem cellstudies, the amount of stem cells injected, if the study used lithium,

and if the effects were positive or negative. As demonstrated in Table 4, there are promising stem cell therapies using lithium is hopeful for the future of the disease. Lithium alone or lithium in addition to an injection of stem cells has demonstrated to delay disease onset and disease progression. Even with positive results, research should be continued in the human clinical trials of therapy for ALS.

Stem Cells	Amount of Stem Cells Transplant	Lithium?	Response (+/-)
Neural Stem Cells	100,000	No	Positive
Neural Stem Cells	20 injections of 400,000 fetal spinal cord NSCs	No	Negative
Human Bone Marrow	1,000,000	No	Positive
Mesenchymal Stem Cells	15,000,000	No	Positive
Human Bone Marrow	8,500,000	Yes	Positive
Induced pluripotent stem cells	40,000	No	Positive
Cord blood	2,500,000	No	Positive

Table 4: Represents discussed stem cell therapies, the amount of stem cell in a transplant, the addition of lithium, and if the response to the stem cells were positive or negative throughout the paper.

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