Emerging Therapies in Amyotrophic Lateral Sclerosis

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Abstract
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, characterized by the loss of cortical and spinal motor neurons, leading to weakness, muscle atrophy, and, in a substantial number of patients, cognitive impairment. Most patients die within 2 to 5 years of diagnosis. The disease initiates from the death of upper and lower motor neurons leading to a degeneration of motor pathways and the paralytic effects of the disease. The disease has huge economic costs as well. FDA has approved two drugs, Riluzole and edaravone, for the treatment of ALS, and Masitinib is being considered for approval. However, these drugs provide modest benefits in mortality and/or function. Recent developments in understanding the underlying pathophysiologic processes that contribute to ALS have led to the development of numerous investigational therapies with several molecules in phase 3 trials. This article highlights the epidemiology, pathophysiology, and several current and emerging treatment options for ALS, including stem cell therapy.

Keywords: Amyotrophic lateral sclerosis, neurodegenerative, treatments. stem cell therapy, muscle atrophy

Introduction
Amyotrophic Lateral Sclerosis is a progressive motor neurodegenerative disease that is characterized by a loss of motor function in muscles under voluntary control. The first case of ALS was reported in the mid-nineteenth century and was identified by French neurologist Jean-Martin Charcot (Amyotrophic Lateral Sclerosis (ALS) Fact Sheet | National Institute of Neurological Disorders and Stroke, n.d.). However, it was not widely known until it was diagnosed in a famous baseball player Lou Gehrig after which it became known as Lou Gehrig’s disease. This disease was named after the visible streaks of degeneration observed in the anterior and lateral tracts of spinal cords due to muscular atrophy in inflicted individuals (Magnussen & Glass, 2017). Based on the region of functional loss, it is known as spinal or bulbar onset ALS. Spinal onset, which is the case in more than 70% of the ALS population (Shellikeri et al., 2017), leads to death within 3 to 5 years from diagnosis, starting as muscle weakness due to muscular atrophy and progressing to impaired muscle function in limbs. On the other hand, survival in cases of bulbar onset ranges from 2 to 3 years. It is the phenotypic expression in remaining patients. However, 85% of the spinal onset cases later present with difficulty in eating, and it starts as an inability to swallow, which then progresses to dyspnea and respiratory paralysis, which ultimately leads to death. According to primitive studies, ALS was considered a subset of Motor Neuron Diseases; however, research in the previous decade suggests that it is a system failure rather than the failure of specific motor neurons. It is known that the cells of nervous origin, in congruence with lymphocytes, pericytes, and interneurons contribute to the decline in energy homeostasis in affected individuals, as confirmed by modern imaging and spectroscopic techniques (Casas et al., 2016). The incidence of ALS can be sporadic or familial. Less than 10% of ALS cases are familial, meaning in less than 10 percent of the cases, there is an
underlying genetic mutation that leads to neuronal degeneration or phenotypic expression of the disease (Boylan, 2015). 90% of the cases are sporadic; there is not an established cause of disease onset. Symptoms of both familial and sporadic ALS include muscle weakness, fatigue, and loss of control in voluntary muscles. Limb onset ALS presents asymmetrical distal weakness and brisk reflexes in a non-functioning limb. Loss of pain sensation and other sensory reflexes, and the most important is that these symptoms tend to get worse during follow-up because it is a progressive neurodegenerative disease. In Respiratory onset ALS, symptoms such as breathing difficulties are observed, and usually, it is in the later stages that the patient suffers respiratory paralysis (McDermott & Shaw, 2008). In more than 50% of cases of ALS, cognitive and behavioral impairment was observed, and it was attributed to the presence of inclusion bodies, usually TAR-DNA binding proteins (TDP-43) in the Frontotemporal lobe. Neuronal degeneration and gliosis were also observed in the said region, which was interpreted as a cause of cognitive and behavioral impairment (Shellikeri et al., 2017). Two major theories explain the pathogenesis in cases of ALS. The dying forward hypothesis explains that neuronal degeneration and death begin from cortical motor neurons that connect to the anterior horn of the spinal cord via a single synapse; in such neurons, degeneration occurs via glutamate excitotoxicity. Upper motor neurons (UMNs) are affected mainly in the dying forward hypothesis. At the same time, the dying-back hypothesis talks about the decline in motor neurotrophic factors in muscles leading to anterograde transport of toxic components to the axonal cell body, which leads to degeneration of lower motor neurons (LMNs). According to Charcot’s explanation, there is more acceptance for the forward dying hypothesis, which explains the underlying mechanisms of neuronal decay, instead of the dying back hypothesis, which talks about anterograde transport of noxious stimuli. However, there is no definitive pathogen diagnosed as well as the degenerative mechanisms of LMN’s are typical for poliomyelitis and Kennedy’s disorder as well hence it is still debatable. An independent dying hypothesis suggests a simultaneous degeneration of UMN’s and LMN’s in cases of ALS, and diagnosis is made based on El Escorial Criteria or Airlie House criteria. The presence of UMN or LMN symptoms or coincidence of both and focal or peripheral presentation of symptoms determine the stages of ALS (Van den Bos et al., 2019). The Awaji-shima criteria for diagnosis of ALS in which needle EMG is used to determine nerve conduction potentials for specific limbs (De Carvalho et al., 2008). El Escorial criteria classify the patients based on the occurrence of UMN or LMN symptoms in one or more than one region as probable or definite ALS diagnosis. Figure 1 shows how the diagnosis of ALS is made certain however, it takes approximately 12 months of progressing symptoms to reach a definite diagnosis. In addition to figure/ Airlie House criteria to rule out the possibility of other LMN diseases with similar symptoms, we use Awaji-shima criteria. Randomized Controlled trials conducted in the last decade have proven the efficacy of Riluzole and Edaravone in treating ALS. There are ongoing clinical trials to prove the efficacy of newer drugs that delay the disease progression and increase life expectancy in one way or the other. In this review, we will discuss in detail the efficacy of Riluzole, Edaravone, and other drugs that have proven to be efficacious in treating ALS (Jaiswal, 2019).

**Epidemiology**

Disease burden across populations is studied to understand probable risk factors for the incidence of ALS. In this section, we will briefly review epidemiologic studies from 1995 to 2020 to get a clear picture of how disease prevalence has changed. According to research published in Clinical Neuroscience Journal in 1995, ALS occurred in the mid-40s, and the incidence ratio for men was higher than that of women.10 percent of the cases diagnosed at the time were familial ALS, and the genetic mutation underlying disease occurrence was found out to be in Cu/Zn SOD1 (Nelson, 1995). A. Chio and associates conducted a ten-year prospective study on 1347 residents from Piemonte and Valle d’Aosta to estimate the incidence of ALS in neurology departments of hospitals in these states. Diagnosis of ALS was based on El Escorial Criteria-Revised. 1260 residents out of the 1347 residents were identified as definite cases of ALS per their symptoms, out of which there were 687 men and 573 women. The mean annual incidence rate was found to be 2.90 per 100,000 population with a 95% Confidence Interval. The incidence rate did not vary considerably during the entire ten years of study and was constantly higher in
Figure 1. El Escorial criteria for diagnosis of ALS. Weakness, hyperreflexia, and spasticity progress over time. EMG, MRI, and Muscle Biopsies are performed to rule out other Motor Neuron Diseases ("Fig. 4. The Revised El Escorial Criteria for the Diagnosis Of..." n.d.).

men than women. Cases were more in the 70-74 age group of men and 75-79 age group of women with men to women ratio of 1.28:1. The study concluded that the incidence of ALS in western countries and Italy was relatively homogenous with a stable onset from 1995 to 2005 (Chio et al., 2009). A prospective study in the Emilia Romagna Region (ERR) of northern Italy from January 1, 2009, to December 31, 2011, included 347 new cases of ALS. Patients of ALS were mostly agriculture workers and were from low-density populations. However, the number of patients was insufficient to look for environmental factors responsible for disease occurrence. Seventeen neurological departments in ERR collected follow-up data and deaths of patients during the study. Results showed that two-thirds of the cases of ALS were spinal-onset cases, 38.33% were of classical phenotype, 34.58% of bulbar, and 19.30% of flail phenotype. UMN and respiratory onset were merely less than 10% of the cases. According to statistics, it was found that the ratio of spinal onset was higher in men, and that of bulbar onset was higher in women. A study showed that Bulbar and Spinal onset had a similar incidence in late ALS; however, early ALS was usually spinal onset. The incidence rate of ALS was classified according to the population densities of different municipalities. In areas with a population density of <50 people/km² incidence rate was 3.27/100,000, whereas in municipalities of ≥50 people/km² incidence rate was 2.55/100,000, with an increasing slope from areas of low density to those of high-density population. The study confirmed the previous finding that it occurred predominantly in males; however, the link to environmental factors was still unclear, even though there were an 8.3% incidence cases of ALS in agricultural workers, but the data is not statistically sufficient to form a conclusion. Genetics alone cannot solve the mystery of the pathogenesis of ALS, so research needs to be conducted in areas where an...
environmental risk factor accounts for disease occurrence in many people (Mandrioli et al., 2014). According to the most recent publications, the trend of male to female relative risk was found to be 0.76 to 1 in the Scottish population, which is contrary to previous research findings in Europe (Leighton et al., 2019). The standardized male-to-female ratio in recent findings of Italy is 1.05 to 1, which shows there is an equal possibility of incidence of ALS in both genders (Palese et al., 2018). This conclusion is supported by other researchers; however, it varies from one geographical area to another based on genetics and environmental factors (Longinetti & Fang, 2019).

Age at incidence differs in Europe from that of Scotland and China, which is between 51 to 66 years on average (Longinetti & Fang, 2019). Greater age at onset in Europe can be because of differences in population designs under consideration, as the age of onset is usually in the mid-sixties. ALS studies have shown an increased Odds Ratio of disease in individuals working in the agriculture sector or exposed to metals such as mercury, selenium, and lead. Occupational exposure to pesticides and exposure to Beta Methyl amino-L-Alanine (BMAA), a neurotoxin produced by cyanobacteria, is another potential environmental risk factor for disease occurrence (Filippini et al., 2020).

Table 1: Potential susceptibility genes for developing ALS (Warner 2009).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene (OMIM)</th>
<th>Localization</th>
<th>Variant</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurofilament heavy chain</td>
<td>NEFH (162230)</td>
<td>22q12</td>
<td>Deletions</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Vascular Endothelial Growth Factor</td>
<td>VEGF (192240)</td>
<td>6p12</td>
<td>Promoter SNPs</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Survival of Motor Neuron 1</td>
<td>SMN1 (600354)</td>
<td>5q12.2-q13.3</td>
<td>Copy number</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Survival of Motor Neuron 2</td>
<td>SMN2 (601627)</td>
<td>5q12.2-q13.3</td>
<td>Copy number</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>APOE (107741)</td>
<td>19q13.2</td>
<td>e4 genotype</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Glutamate Transporter</td>
<td>EAAT2 (600300)</td>
<td>11p13-p12</td>
<td>Decreased expression</td>
<td>Familial, Sporadic</td>
</tr>
<tr>
<td>Glutamate Receptor</td>
<td>GLUR2 (138247)</td>
<td>4q32-q33</td>
<td>Altered RNA editing</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Ciliary Neurotrophic Factor</td>
<td>CNTF (118945)</td>
<td>11q12.2</td>
<td>Null allele</td>
<td>Familial</td>
</tr>
<tr>
<td>Angiogenin</td>
<td>ANG (105850)</td>
<td>14q11.2</td>
<td>SNPs and missense mutations</td>
<td>Familial, Sporadic</td>
</tr>
<tr>
<td>Hemochromatosis gene</td>
<td>HFE (235200)</td>
<td>6p21.3</td>
<td>SNPs</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Spastin</td>
<td>SPG4 (182601)</td>
<td>2p22-p21</td>
<td>Duplication Mutation</td>
<td>Sporadic</td>
</tr>
</tbody>
</table>

Pathophysiology
Loss of motor neurons in the ventral horn of the spinal cord, brainstem, and motor cortex account for the pathogenesis of ALS alongside the presence of disarrayed neurofilaments and ubiquitinated proteins under histological analysis. The selective neuronal degeneration cause cannot be pinpointed. However, several reasons include abnormal proteins, dysfunctional neurofilaments and mitochondria, oxidative damage, and elevated neuroinflammation. Mostly missense point mutations and sometimes nonsense mutations and deletions on the same gene can account for mutations in the SOD1 gene, which is a
prominent cause of Familial ALS in 3% of cases with no history of ALS. Mutation in exon 1, Ala4Val, is responsible for the early-onset rapidly progressing disease, whereas late-onset and slowly progressing diseases are attributable to His46Arg mutation. Around 170 types of mutations can occur in this case, and the possible pathogenesis reported is enhanced enzyme activity that leads to protein misfolding and oxidative stress. Several other mutations are responsible for sporadic and familial ALS, as shown in Table 1 (Warner, 2009). Around 39% of familial ALS patients and 7% of sporadic ALS patients have C9ORF72 mutation, which presents in the form of multiple nucleotides repeat units of six nucleotides in the sequence GGGGCC in Intron 1 or promotor of the gene. This happens at sense and anti-sense strands, ultimately leading to the formation of five abnormally functioning proteins. Forty-three mutations of TAR-DNA binding proteins are found in 5% of the individuals affected by ALS. TDP-43 is an RNA and a DNA binding protein involved in transcription, mRNA splicing, transport, and stability. Pathogenicity could be because of a loss or gain in the function of the TARDBP gene that could lead to disruption of stress granules and accumulation of ubiquitinated inclusions in the cytoplasm, while on the other hand disrupting nuclear RNA metabolism. FUS (fused in sarcoma) is found in around 5% of patients affected by ALS, and its mechanism of pathogenesis is similar to that of TDP-43 (Morgan & Orrell, 2016). Figure 2 shows different molecular pathways that account for pathogenesis in ALS.

**Figure 2.** Complex molecular and genetic pathways account for the pathogenesis in ALS. Dysfunction of the excitatory amino acid transporter 2 on astrocytes leads to reduced uptake of glutamate, resulting in glutamate excitotoxicity which further induces neurodegeneration by stimulation of Ca$^{2+}$ dependent enzymatic pathways. Mutations in C9ORF72, TARDBP, and FUS genes contribute to the dysregulated RNA metabolism and result in the accumulation of misfolded neuronal aggregates. Increased oxidative stress is attributed to mutations in the SOD1 gene. Activated microglia secrete proinflammatory cytokines and cause neurotoxicity.
Treatment Approaches
Edaravone was developed in the early 1990s as an anti-epileptic free radical scavenging agent for stroke. However, it was never approved for its clinical management in Europe and USA, but it was approved in Japan in 2001. From 1990 to 1995, phase 3 trials were completed for Riluzole, and it was approved by FDA for ALS in 1995. Lithium, Vitamin E, Pioglitazone, and Minocycline were added to Riluzole therapy, and their synergistic effects were to be observed. No significant improvement in symptoms was observed with these combinations. Edaravone entered phase 3 clinical trials and was approved for ALS subsequently in Japan and USA. Induced pluripotent stem cell trials and stem cell transplant trials are being conducted in the hope of improving survival in ALS patients. Figure 3 shows the site of action of various chemotherapeutic and non-chemotherapeutic agents being used in treating ALS (Jaiswal, 2019).

Riluzole
Riluzole, chemically known as 2-Amino-6-(trifluoromethoxy) benzothiazole, acts as an anti-glutamatergic and anti-excitotoxic drug. Its precise mechanism in the treatment of ALS is not known; however it acts by inhibiting glutamate release, inactivates voltage-dependent sodium channels, and it interferes with the binding of neurotransmitters to excitatory amino acid receptors. In the case of ALS, excessive glutamate release in motor neurons leads to elevated Ca\(^{2+}\) levels in glial and soma cells. Elevated intracellular calcium levels lead to peroxidation of membrane lipids, disruption of RNA and DNA and lead to dysregulation of mitochondrial function and lead to cell death, and production of superoxide anion and hydrogen peroxide, which lead to a continued chain reaction and lead reaction within cell components such as proteins, lipids, and DNA (Jaiswal, 2019).

Edaravone
Edaravone primarily acts as a ROS scavenger, as a neuroprotective drug. A study on mice showed that it slowed motor neuron degeneration and a decline in motor symptoms. An open-label phase 2 clinical study has proven a decline in oxidative radical levels as measured by the levels of 3-nitrotyrosine in the treated group. Subsequently, a double-blind placebo-controlled study was conducted over 24 weeks and did not show the marked effects of Edaravone in treating ALS. However, the study protocols required the monitoring duration to be within 12-18 months, so we can stick to the FDA approval for using Edaravone for improving motor function, which is also reinforced by a reduction in oxidative stress (Hardiman & Berg, 2017).

Masitinib
Masitinib is a tyrosine kinase inhibitor, and it has a neuroprotective effect in addition to immunomodulation for which it was used in a rat model of ALS that had SOD1 mutation, and the study results proved beneficial in them. Masitinib was used as add-on therapy with Riluzole, and its effect was observed in 394 patients in a double-blind, placebo-controlled randomized trial, in which patients were given Riluzole (100mg/day) and placebo or masitinib. In this study masitinib was given in 3.0 mg/kg/day or 4.5 mg/kg/day. The study results confirmed the tolerance of masitinib at both doses. The primary endpoint was a decline in deteriorating function on ALSFRS-R after 48 weeks of study. The ALSFRS-R score gained 3.4 points, and improvement in function was observed in the treated group. This corresponds to a 27% improvement in functional decline. A phase III double-blind study is going to be started soon, and 500 patients will be part of this multicentric double-blind, placebo-controlled, parallel groups study (Mora et al., 2020).

Tauroursodeoxycholic Acid (TUDCA)
Neuroprotective action on motor neuron-neuroblastoma cells having SOD1 mutations A4V and G931A was observed against NO toxicity. Previously TUDCA was well known among gastroenterologists for its use in treating gall stones and cholestatic liver diseases. In the case of ALS, it is being used owing to its potent inhibition of apoptotic pathways in mitochondria leading to a diminished caspase activity and reduced production of oxygen radicals (Amaral et al., 2009). A Phase II study has previously shown promising results in terms of safety and tolerability of the dose of UDCA as given by the improvement in score in terms of ALSFRS-R criteria, 87% of the patients enrolled responded to the treatment, and adequate CSF levels were monitored (Elia et al., 2016). A phase III clinical trial is being conducted in Europe, and 440 participants are going to be enrolled in the double-blind, placebo-controlled randomized study in which patients will be receiving 2 g TUDCA in addition to 50
mg Riluzole for 18 months, and the change in ALSFRS-R score will be observed every three months during the study (NCT03800524) (Humanitas Mirasole SpA, 2021).

Rho-kinase Inhibitor
Rho-kinase inhibitor (ROCK), Fasudil, has shown commendable results in treating neuropathic pain, subarachnoid hemorrhage, cerebral stroke, epilepsy, and Parkinson’s disease (Nizamudeen et al., 2018). ROCK is a serine/threonine kinase inhibitor and is expressed in two isoforms, ROCK1 is expressed more peripherally while ROCK2 is expressed greatly in CNS (Lingor et al., 2019). Elevated levels of Rho-kinase can lead to neuronal degeneration, oxidative stress cytokine release, and protein accumulation, which are some of the common presentations of neurodegenerative diseases (Koch et al., 2018). A multicenter, double-blind, randomized, placebo-controlled phase IIa study will be conducted across 16 centers in Germany, Switzerland, and France, recruiting 120 patients that have definite or probable ALS (according to El Escorial criteria). They will be administered with doses of 30mg/day or 60mg/day or a placebo for 20 days, and results will be observed after 90 and 180 days. The primary end-point of the study will be the safety and tolerability of the administered drug (Lingor et al., 2019).

Tamoxifen
Tamoxifen is an FDA-approved drug for treating cancer by binding to estrogen receptors; however, it has shown neuroprotective function by autophagy of TDP-43 inclusions in the cytoplasm, which is a characteristic pathology of ALS. Tamoxifen 40 mg/day was administered to the patients in addition to Riluzole. The trial did not show a very marked difference in the improvement of function. To be more certain about the effect of tamoxifen, a study with a greater number of patients must be conducted (Chen et al., 2020).

Samples were collected in ALS patients at the time of diagnosis, and elevated serum ferritin levels and diminished transferrin levels were observed in 104 Sporadic ALS patients in comparison to 145 controls (Veyrat-Durebex et al., 2014). In the post-mortem studies, anti-ferritin was used that densely stained the cortex. The Susceptibility-weighted Imaging (SWI) showed the deposition of iron in the motor cortex of ALS patients compared to the normal control group (Adachi et al., 2015). An improvement in survival by 56% was observed in female mice in a dose and sex-dependent deferiprone study conducted on mice when 50mg/kg/day was administered. Similarly, 100mg/kg/day and 200mg/kg/day were also administered, and less improvement was observed in the former, and no improvement was observed in the latter doses. In male mice, a 200mg/kg/day dose was shown to be effective only (Moreau et al., 2018). FAIR-ALS II, an interventional study of deferiprone is being conducted in France, 210 participants have been enrolled in this study, and they are to be given 600 mg delayed-release deferiprone at 30mg/kg/day, and the other group will get a placebo twice daily for 12 months (NCT03293069) (Conservative Iron Chelation as a Disease-Modifying Strategy in Amyotrophic Lateral Sclerosis - Tabular View - ClinicalTrials.Gov, n.d.).

Levosimendan
Levosimendan, a calcium sensitizer, was given to normal healthy subjects, and an improvement in contractile function and neuromechanical efficiency was observed in the human diaphragm. Improvement in respiratory function was observed in REFALS.REFALS is a double-blind, placebo-controlled randomized phase III clinical trial conducted in 99 ALS centers across 14 countries. Four hundred ninety-six people were made part of the study, out of which 329 were randomly assigned to receive levosimendan, and 146 participants received a placebo. The study results did not show a remarkable difference in Slow-vital capacity in patients treated with ALS. Further studies are being conducted to evaluate the role of levosimendan in ALS treatment (Cudkowicz et al., 2021).

Non-chemotherapeutic Approach
Food Intervention Studies
Malnutrition is a prognostic factor in ALS, an interhospital study conducted in Spain compared individuals with higher calorie intake to the ones who were undernourished. The study results showed better survival in patients with high-calorie intake (López-Gómez et al., 2021). A double-blind, placebo-controlled, randomized phase 2 clinical trial conducted in the US showed the safety and tolerability of a tube-fed high-calorie diet in ALS patients. The Control group received an iso-caloric tube-fed diet, and the test groups either received a high-carbohydrate hypercaloric tube-fed diet or a high-fat hypercaloric tube-fed diet. The
The primary end-point of the study was the safety and tolerability of the high caloric enteral intake in ALS patients during the early stages of the disease (NCT00983983) (Wills et al., 2014).

**Rovelizumab**

Monoclonal antibodies are usually used for treating auto-immune disorders. Their use in ALS came to notice after a thorough study by researchers on the altered levels of complement pathway proteins during disease progression in humans and animal models. The inappropriately activated complement pathways play a crucial role in the pathophysiology of ALS as dysregulation of ubiquitous complement proteins. Increased C3 and C4 levels in blood and CSF of ALS patients confirm the role of the complement pathway in neuroinflammation. C5 is expressed by motor neurons responsible for auto-degeneration in addition to C5RA and CD88 from microglia (Kjældgaard et al., 2018). Rovelizumab acts by inhibiting the activation of C5 into C5A and inhibiting the activation of complement cascade leading to slowed disease progression as confirmed in mouse models. An open-label, double-blind, placebo-controlled, randomized phase 3 clinical trial is being conducted in France to check the efficacy of rovelizumab in diminishing the progress of ALS, and the efficacy will be determined using the ALSFRS-R scale (Unpublished Study) (NCT04248465).

**Stem-Cell Therapy**

Multi-potential stem cells act by producing growth-stimulating (neurotrophic factors) that migrate to the damaged neuronal sites and stimulate the regenerative

Figure 3. Action sites of chemotherapeutic and non-chemotherapeutic moieties. Edaravone and Riluzole are the only FDA-approved drugs for treating ALS. Tamoxifen, Masitinib, TUDCA, Deferiprone, Fasudil, and Levosimendan are undergoing trials for their safety and efficacy. Rovelizumab and Stem Cell therapy are also being studied for their therapeutic benefit in ALS.
process in neurons. Autologous (a person’s own) bone marrow and peripheral cells have been used in multiple trials of neurodegenerative diseases because they have minimal chances of rejection (Wahid et al. 2019).

A phase-2 RCT was conducted in Tertiary Care Centre for ALS in Korea in which 64 patients participated. The patients’ groups were divided into 2, 31 patients received Riluzole alone, and the other group (33 patients) received 2 Bone marrow- Mesenchymal Stem Cells intrathecal injections. The outcome was measured as an improvement in functionality using the ALSFRS score. The study showed no marked difference in the occurrence of side effects in patients of both groups. Safety and efficacy were proven for six months. A decline in ALSFRS score in autologous BM-MSC injections indicates the efficacy of these injections. (NCT01363401) A phase 3 study is going to be conducted to determine the safety of stem cell therapy in the long term (Oh et al. 2018).

Human Neural Stem Cells were extracted from an 8-week baby and were used to test the effect of NSCs in SOD1 G93A mutated mice models. Neural Stem Cells were transplanted in the Cervical and Lumbar regions from where they migrated to the spinal cord grey and white matter after differentiation. The disease onset was delayed by ten days in rats that were administered Live NSCs in comparison to the ones that got dead NSCs. Also, the survival time was increased by 17 days in rats that were transplanted with live NSCs. These results proved to be significant on a t-test. Stem cell therapy proves to be a promising approach to treating ALS (Xu et al. 2011).

**Discussion and Conclusion**

A deeper understanding of the molecular processes that contribute to the pathogenesis of ALS has led researchers to explore more investigational drugs that were previously used in the symptomatic treatment of motor neuron diseases. A variety of supplements and antioxidants were claimed to be beneficial in alleviating disease progression, but due to lack of scientific evidence, they are not being used in treating ALS except for methylcobalamin, which is shown to reduce homocysteine levels in the nervous system that accounts for neurotoxicity in ALS. A phase 3 clinical study was conducted to prove the efficacy of Methylcobalamin in treating ALS, showing a delay in functional decline (NCT03548311). Edaravone and Riluzole are the only FDA-approved drugs for the treatment of ALS, and Masitinib is being considered for approval owing to its neuroprotective role in glial cells. NU-9 is an investigational drug that has shown improvement in axon health of upper motor neurons in two ALS mouse models; it showed improvement in axonal health within 60 days, so much so that the diseased neurons resembled control neurons. Safety and toxicity studies are being conducted on this drug, and after the completion of these studies, clinical trials will be started in early 2023 (Marla Paul 2022). Stem cell therapy has shown promising results in mouse models, and phase 3 studies are underway to prove their effects in the long term. Reldesemtiv is another investigational drug currently under observation for its therapeutic activity in ALS patients (NCT04944784) (Unpublished data). Microbiome role in disease progression in ALS is also being studied, and the link between a deficiency in certain species linked to disease progression is going to be reported in ongoing studies. There is still considerable evidence required for determining the efficacy of the above-mentioned treatment options in controlling disease progression in ALS.

**Conflict of Interest**
The authors declare that they have no competing interests.

**Funding**
There was no specific funding available for this project.

**Study Approval**
NA

**Consent Forms**
NA.

**Authors Contribution**
LF conceptualized the study and wrote the final manuscript, LF and MT helped in the literature search, curation and analysis and wrote the final manuscript. MT made all the illustrations present in this manuscript.

**Acknowledgments**
The authors thank the management of the Shifa Tameer-e-Millat University for encouraging this scholarly activity.

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