The role of TDP-43 protein in amyotrophic lateral sclerosis

Piotr Włodarczyk  
Faculty of Medicine, Poznan University of Medical Sciences, Poland  
Corresponding author: eedris888@yahoo.com

Mikołaj Witczak  
Faculty of Medicine, Poznan University of Medical Sciences, Poland

Agnieszka Gajewska  
Faculty of Medicine, Poznan University of Medical Sciences, Poland

Tomasz Chady  
Faculty of Medicine, Poznan University of Medical Sciences, Poland

Igor Piotrowski  
Department of Electroradiology, Poznan University of Medical Sciences, Poland; Radiobiology Laboratory, Department of Medical Physics, Greater Poland Cancer Centre, Poland  
https://orcid.org/0000-0002-4985-9321

Keywords: Amyotrophic lateral sclerosis, ALS, TDP-43, SINE, Antisense oligonucleotides, Chaperones, autophagy enhancers

Published: 2022-12-30

How to Cite: Włodarczyk P, Witczak M, Gajewska A, Chady T, Piotrowski I. The role of TDP-43 protein in amyotrophic lateral sclerosis. Journal of Medical Science. 2022;91(4);e710. doi:10.20883/medical.e710

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease where both upper and lower motoneurons are damaged. Even though the pathogenesis of ALS is unclear, the TDP-43 aggregations and non-nuclear localization may be crucial to understanding this process. Despite intensive research on ALS therapies, only two lifespan-prolonging medications have been approved: Riluzole and Edaravone. Unravelling the TDP-43 pathology could help develop new ALS therapies using mechanisms such as inhibition of nuclear export, autophagy, chaperones, or antisense oligonucleotides. Selective inhibitors of nuclear export (SINEs) are drugs that block Exportin 1 (XPO1) and cause the accumulation of not exported molecules inside the nucleus. SINEs that target XPO1 are shown to slightly extend the survival of neurons and soften motor symptoms. Dysfunctional proteins, including TDP-43, can be eliminated through autophagocytosis, which is regulated by the mTOR kinase. Stimulating the elimination of protein deposits may be an effective ALS therapy. Antisense oligonucleotides (ASOs) are single-stranded, synthetic oligonucleotides that can bind and modulate specific RNA: via ribonuclease H, inducing their degradation or inducing alternative splicing via blocking primary RNA transcripts. Current ASOs therapies used in ALS focus on SOD1, C9ORF72, FUS, and ATXN2, and they may be used to slow the ALS progression. Reversing the aggregation is a promising therapeutic strategy. Chaperones control other proteins’ quality and protect them against stress factors. Due to the irreversible character of ALS, it is essential to understand its complicated pathology better and to seek new therapies.
Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehring’s disease, or Charcot’s disease, is a progressive neurodegenerative disorder leading to the loss of motor neurons. Limb and bulbar onset are ALS’s most common clinical phenotypes, responsible for 70% and 25% of all cases. Signs of upper and lower motor neuron (UMN and LMN) damage are required to confirm the diagnosis of ALS. UMN disruptions are presented with spasticity and weakness, in contrast to LMN malfunctions manifested by fasciculations, muscle wasting, and weakness. Dysarthria and dysphagia are bulbar signs of ALS [1]. The progressive character of ALS leads to malnutrition and respiratory failure. In Europe, the incidence of ALS varies between 2.1 and 3.8 per 100,000 person-years (2019, review) [2]. The median age of ALS diagnosis is between 54 to 69, and the median time from first symptoms to diagnosis ranges between 9 and 24 months [2]. The median survival time from the first symptoms to death or invasive respiratory ranges between 24 and 50 months [2]. However, 5–10% of patients live longer than 10 years. Older age, bulbar onset, malnutrition, psychological distress, lower forced vital capacity (FVC), and the short time delay between onset and diagnosis are related to worse clinical outcomes. [3] 30–50% of ALS patients show cognitive function deficits, and 15% meet the criteria for frontotemporal dementia (FTD) [4]. In addition, ALS and FTD share a common neurological hallmark: up to 97% of ALS and 45% of FTD patients’ nervous systems have TDP-43 positive neuronal aggregates [5]. These findings support the hypothesis that ALS and FTD are two manifestations on an ALS-FTD spectrum.

The direct cause of ALS is unknown, but 10–15% of patients have a positive family record. More than 30 genes have been identified as risk factors for ALS development. Almost 70% of familial ALS (fALS) cases are associated with the mutations in superoxide dismutase 1 (SOD1), fused in sarcoma (FUS), chromosome 9 open reading frame 72 (C9ORF72), TAR DNA-binding protein 43 (TARDBP) [6, 7]. Environmental factors may relate to the more frequent onset of the sporadic form of ALS. Exposure to pesticides or low-frequency electromagnetic fields induces cellular oxidative stress, which could contribute to the pathogenesis of many degenerative diseases [8]. Exposure to heavy metals like lead, head trauma, professional sports, intensive physical activity, and lower body mass index are all associated with a higher probability of developing ALS [8, 9]. However, the research presenting this relationship shows that the estimated probability was low. Additionally, the number of studies on environmental factors is relatively modest. Consequently, these factors are poorly established.

Although the pathomechanism of ALS is unclear, the impaired TDP-43 protein plays a crucial role in the pathogenesis of the disease. TDP-43 is a highly conserved protein belonging to the family of DNA/RNA binding ribonucleoproteins. It is mainly located in the cell nucleus [10]. In most ALS cases, TDP-43 is mislocalized from the nucleus to the cytoplasm, simultaneously forming ubiquitinated cytosolic aggregates [10, 11]. Due to ALS’s progressive and irreversible character, it is essential to seek new therapies to improve patients’ prognoses. Nevertheless, only two disease-modifying medications have been approved: Riluzole (possibly inhibiting glutamatergic transmission) and Edaravone (free radical scavenger; cleared by FDA but withdrawn by EMA) [12].

In this article, we present the role of impaired TDP-43 protein in the pathology of ALS and discuss current (riluzole and edavarone) and emerging ALS therapies using such methods as inhibition of nuclear export, autophagy enhancement, chaperones, antisense oligonucleotides, and inhibition of poly (ADP-ribose) polymerase.

Methodology

Papers published between 1995 and June 2022 were identified by PubMed literature searches using the terms: “amyotrophic lateral sclerosis”; “ALS”; “TDP-43 proteinopathy”; “antisense oligonucleotides”; “autophagy enhancers”; “chaperones”; “SINE”; “edavarone”; “riluzole”. Additional publications were selected through the Internet from the references of those papers. Only articles in English were considered.

TDP-43 protein biology

TDP-43 is a nuclear protein encoded by the TARDBP gene located on chromosome 1. It was first recognized as a protein that binds to the trans-
activation response (TAR) element of the human immunodeficiency virus (HIV) and thus was named TAR DNA-binding protein-43 kDa [10]. TDP-43 is mainly concentrated in the nucleus but may perform some of its functions in the cytoplasm [13]. The primary function of the TDP-43 is RNA metabolism which includes its transcription, translation, messenger RNA (mRNA) transport and stabilization, micro RNA (miRNA), and long non-coding RNA (lncRNA) processing [14].

TDP-43 is involved in forming and regulating the cytoplasmic RNA granules, termed stress granules (SGs), that appear after exposure to environmental factors, including oxidative or osmotic stressors, heat shock, or viral infections [15]. These membrane-less organelles are thought to enhance cell survival through by storing mRNAs, translation factors, and RNA-binding proteins following stress exposure. Additionally, TDP-43 residues may be significant in SGs formation in the liquid-liquid separation process [16]. Liquid-liquid phase separation of RNA-binding proteins, such as TDP-43, is a process in which membrane-less organelles are formed in cells. The abnormal phase transition of these proteins leads to the formation of insoluble protein aggregates [17]. TDP-43 proteinopathy identified as a factor in the pathogenesis of ALS and other neurodegenerative diseases develops through the depletion of the TDP-43 protein in the nucleus with its mislocalization and aggregation in the cytoplasm [18, 19]. The surge in the cytoplasmic TDP-43 concentration leads to cytoplasmic aggregation formation observed in ALS [20]. Studies suggest that the cytoplasmic mislocalization of TDP-43 induces toxicity through both the loss and gain of functions [10].

The protein consists of 414 amino acid residues composing four domains: N-terminal (NTD), two highly conserved RNA-binding domains (RRM) as well as an unstructured Carboxyl-terminal fragment (CTF) [18]. TDP-43 RRM domains bind with related RNA/DNA molecules and are involved in RNA metabolic processes [19]. Studies suggest that the RNA binding ability may be a toxic and protective mechanism of TDP-43 protein during its aggregation [19, 21]. Several mutations in the RRMs were shown to disrupt the RNA binding capability while not significantly interfering with RNA recognition [22]. NTD is responsible for the interactions of partner proteins and the target RNAs and may protect against cytoplasmic TDP-43 aggregation [23,24]. Deletions or mutations in the nuclear localization signal (NLS) sequence of the NTD may cause the mislocation of TDP-43 in the cytoplasm [25].

TDP-43 aggregates in patients include its full-length form and the 35- and 25-kDa CTF, the prion-like structure of the CTF that is most important for TDP-43 neurodegenerative properties, as CTF is the dominant ALS-associated TARDBP mutation site [26–28]. Prions are self-replicating proteins that undergo conformational changes to form aggregates causing neurological infectious diseases in mammals [29]. While ALS is not an infectious disease, the misfolded structure of TDP-43 and its ability to aggregate give it prion-like properties. Moreover, there is evidence that TDP-43 aggregations can self-propagate within neuronal cells and transmit to adjacent cells. This mechanism, similar to prion replication, may be a foundation for the pathology of ALS [30].

The hyperphosphorylated and ubiquitinated CTF aggregations are found in the brain of patients with ALS. However, CTFs are rarely observed in the spinal cord of ALS patients, even the ones with remarkable degeneration of spinal motor neurons. The above suggests that TDP-43 CTFs accumulate due to additional factors influenced by regional heterogeneity in the central nervous system [29]. TDP-43 accumulation and propagation in vulnerable brain regions and the spinal cord contribute to a significant loss of motor neurons and, thus, clinical syndromes of the neurodegenerative disorder [32]. The wide range of TDP-43 protein cell functions and its post-translational modifications that include ubiquitination, phosphorylation, and acetylation indicate the diversity of biochemical mechanisms in the pathogenesis of ALS itself. (TDP-43 biology is illustrated in Figure 1).

Riluzole

Riluzole is the only medication approved for ALS treatment in Europe [33]. Although initial clinical trials showed that riluzole treatment increases a patient’s lifespan by 2–3 months, a retrospective meta-analysis revealed that survival time could be extended by 6 to even 19 months [34].
Riluzole moderately impacts bulbar and limb function while not influencing muscle strength [35]. Glutamate excitotoxicity is another proposed mechanism that might explain complex ALS pathology; increased glutamate levels in the cerebrospinal fluid of ALS patients support this theory [36]. It was established that riluzole could modulate glutamatergic signaling. However, a direct connection with glutamate receptors has never been shown [36]. Excitatory amino acid transporter-2 (EAAT2) is a transporter responsible for glutamate reuptake, and it is expressed in glial cells. EAAT2 mRNA transcript levels are significantly lower in TDP-43 proteinopathy leading to the accumulation of glutamate [36]. It was suggested that riluzole could inhibit the catalytic activity of protein kinase CK1δ, preventing the formation of hyperphosphorylated TDP-43 aggregation and cytoplasmic mislocalization [36]. The preservation of TDP-43 homeostasis mediated by riluzole enables the correct maturation of mRNA transcripts and, as a result, the typical expression of the EAAT2 transporter. This phenomenon shows a connection between ALS hallmarks: glutamate excitotoxicity and TDP-43 aggregations [37]. Furthermore, riluzole can modulate TDP-43 self-interaction without changing TDP-43 expression; it may result from riluzole’s antioxidative character [33]. Although most ALS patients receive riluzole after diagnosis, a recent study performed on a murine model showed that riluzole administration does not alter total levels of TDP-43 and does not affect the accumulation of TDP-43 aggregations [38]. However, little research shows that riluzole reduces TDP43 protein aggregation. Therefore, inhibition of CK1δ kinase may be a successful approach to reduce TDP-43 aggregation.

**Edavarone – free radical scavenger**

Since 2001 edavarone has been used in the therapy of acute ischemic attack, and the FDA approved it in 2017 for ALS treatment [39]. Edavarone – a free radical scavenger, reduces oxidative stress, a mechanism linked to the pathology of many neurodegenerative diseases that also quickens neuron degeneration [40]. Due to the low number of studies, the direct connection between

---

**Figure 1. TDP-43 biology in ALS**

TDP-43 protein is composed of four domains: N-terminal (NTD), two highly conserved RNA-binding domains (RRM), and a Carboxyl-terminal fragment (CTF). Deletions or mutations in the nuclear localization signal (NLS) sequence of the NTD may cause the TDP-43 mislocation and increased cytosol concentration. Mutations in the RRM domains result in disrupted RNA binding capability. Self-propagation of TDP-43 is mainly the result of the prion properties of CTF. Accumulation of hyperphosphorylated and ubiquitinated CTF-rich aggregations, TDP-43 cytoplasmic mislocation, and increased concentration under stress exposure leads to stress granule formation.
edaravone and TDP-43 alterations needs to be clarified. Nonetheless, the antioxidative character of edavarone might impact the TDP-43 aggregation. 3-nitrotyrosine, a specific marker for oxidative stress connected with neuronal degeneration, was significantly reduced after edavarone treatment in the ALS model [41]. It has been demonstrated that reactive nitrogen species can promote TDP-43 aggregation through S-nitrosylation, leading to the formation of disulfide bonds. Additionally, enhanced nitric oxide generation and protein misfolding are caused by increased TDP-43 expression. This positive feedback loop increases nitrosative stress and protein aggregation [42]. A study conducted by Ohta et al. revealed that edavarone could modulate antioxidant cell response mediated by nuclear factor erythroid 2-related factor-2 (Nrf2) in ALS murine model [43]. Nrf2 controls cell antioxidative response. TDP-43 model demonstrated that in response to oxidative stress, the Nrf2 protein level and the expression of antioxidant genes are increased. However, the levels of glutathione (an essential antioxidant) are reduced [44]. In addition, TDP-43 alters the expression of the RNA-binding protein hnRNP K, resulting in a toxic gain of function. aberrant hnRNP K binds to antioxidant gene transcripts affecting their translation and leading to insufficient antioxidant cell response [44]. ALS patients may find edavarone treatment beneficial by delaying the loss of physical function [45].

Selective inhibitors of nuclear export

Exportin 1 (XPO1) is involved in mediating the export of many types of proteins and RNAs out of the cell nucleus, particularly cargos with leucine-rich nuclear export signals (NES) [46, 47]. Selective inhibitors of nuclear export (SINEs) are drugs that block XPO1 and cause the accumulation of not exported molecules inside the nucleus. SINEs are shown to be helpful in patients with tumours like glioblastoma [48] and multiple myeloma, for which it is approved by the Food and Drug Administration (FDA) to be used as a 5th line of therapy [49]. Using SINEs enhanced autophagy and lysosomal regulation through Helix Loop Helix-30 (HLH-30) nuclear enrichment inSOD-1 based model of ALS in flies and nematodes, thus prolonged their lifespan and prevented neurodegeneration. HLH-30 is an ortholog of human Transcription factor EB (TFEB), a protein responsible for the modulation of autophagy [50]. Therefore, similar effects in ALS-affected patients might be achieved in the future. Some ALS models revealed that SINEs that target XPO1 slightly extend the cellular survival of neurons and soften motor symptoms. However, there was no evidence that one specific inhibitor could influence TDP-43 cytoplasmic levels. To prevent this, several overlapping mechanisms with multiple transporters like XPO7 or Nuclear RNA Factor 1 (NXF 1) have to target the nuclear export [51]. Some studies show that TDP-43 does not contain XPO-1-dependent NES. Therefore, the nuclear egress of TDP-43 is believed to be independent of XPO-1. The TDP-43 protein is suggested to be predominantly size-dependent and driven by passive diffusion [52].

Inhibition of poly (ADP-ribose) polymerase (PARP)

Poly (ADP-ribose) polymerase (PARP) is responsible for protein modification and DNA repair [53]. PARP-1 levels are increasing under oxidative stress conditions. The occurrence leads to an accumulation of ADP-ribose polymers, which eventually may lead to cell death. This phenomenon plays a vital role in developing ALS and other neurodegenerative diseases [54, 55]. Heterogeneous ribonucleoprotein A1 (hnRNP A1) is another RNA-binding protein that also plays a role in the pathogenesis of ALS [56]. Exaggerated response to a stressor such as a reactive oxygen species leads to the recruitment of both hnRNP A1 and TDP-43. Conversely, the decreased Poli ADP-ribosylation (PARylation) suppresses the formation of these stress granules in motor neuron-like cell lines. Therefore using PARP inhibitors, such as a drug like Olaparib used in the treatment of ovarian and breast cancer, may be a candidate for further investigation in ALS treatment [55].

Autophagy induction

Autophagocytosis is a multi-step process responsible for eliminating dysfunctional organ-
elles and proteins, including TDP-43, FUS, TAF15, and EWSR1. Research indicates that impaired autophagy takes part in the formation of protein aggregations in eukaryotic cells such as motoneurons [57]. It may also play a role in the pathogenesis of neurodegenerative diseases. Numerous autophagosomes were found in the motoneurons of patients with both ALS forms, indicating disrupted autophagocytosis [58]. The autophagy pathway is regulated by mTOR kinase, which inhibits the entire process. The use of mTOR inhibitors, and hence the promotion of the elimination of protein deposits, could be crucial for future ALS therapy [59]. Rapamycin (Sirolimus) is a well-known and widely used immunosuppressive drug that stimulates autophagocytosis as an mTOR inhibitor. Ongoing clinical trials indicate controversial effects of Sirolimus in various genetic animal models. Additional improvement in the function of locomotor cells with TDP-43 deposits has d Rapamycin’s protective effect [60]. Trehalose is a natural disaccharide that stimulates rapamycin-dependent autophagy and thus lowers the concentration of TDP-43 in spinal cord cells and motoneurons [61, 62]. It also stimulates the nuclear translocation of TFEB (transcription factor EB), which regulates the genes depending on the autophagy pathway. Trehalose treatment also induces rapid and transient lysosomal enlargement and membrane permeabilization [62]. Lithium is another element that enhances autophagocytosis, and is commonly used in treating mental diseases. Lithium also has a therapeutic effect depending on the patient’s genotype. ALS patients with the UNC13A mutation benefit most from blocking the mTOR kinase pathway [63].

Antisense oligonucleotides therapies

Almost 10% of ALS cases are familial (familial amyotrophic lateral sclerosis, fALS). 70% of fALS could be explained by known mutations, the essential being: SOD1, C9ORF72, FUS, and TAR-DP [64]. The link between known mutations and ALS opens possibilities for personalized medicine, such as using antisense oligonucleotides (ASO). ASOs are single-stranded, synthetic oligonucleotides that can bind RNA with high specificity and could modulate gene expression in two different ways. The first mechanism involves the degradation of targeted RNA mediated by ribonuclease H, which lowers the level of the targeted protein. ASOs may also affect alternative splicing by acting as splice-switching oligonucleotides. Exon skipping and exon inclusions are two forms of splicing modification [64, 65].

Superoxide dismutase is an enzyme encoded by the SOD1 gene. Mutations in SOD1 are 10% fALS and 2% sALS (sporadic amyotrophic lateral sclerosis) cases [66]. However, The connection between the mutation in the SOD1 and the TDP-43 protein is unclear. Some authors suggest no TDP-43 aggregations in SOD1-fALS [67]. Nevertheless, others report that a high concentration of SOD1 protein may affect TDP-43 through phosphorylation and fragmentation, as a result of which SOD1 promotes nuclear-cytoplasmic mislocalization and accumulation of TDP-43 in the cytoplasm [68,69]. Tofersen is the ASO therapy for SOD1-fALS currently in the clinical trial (BIIB067). It is an ASO that binds directly to SOD1 mRNA, stimulating its degradation via RNase H, which prevents the toxic accumulation of SOD1 [70]. Phase 1 and 2 studies have shown that tofersen in a dose of 100 mg reduces the concentration of SOD1 in cerebrospinal fluid by 36% compared to placebo [65]. Additionally, it may slow disease progression, but this requires further studies [71].

Fused in sarcoma (FUS), like TDP-43, belongs to the family of RNA binding proteins (RBP). RBPs are located mainly in the nucleus and are involved in RNA metabolism [672]. The leading cause of early-onset ALS is FUS mutations [73] which are responsible for 4% of fALS and 2% of sALS cases [74]. No TDP-43 deposits were found in FUS-fALS [67]. ION363 is an ASO designed against the 6th intron of the FUS transcript [74]. The first-in-human study showed that repeated administration of ION363 to a FUS-ALS patient reduced of FUS aggregates characteristic for FUS-fALS. The above study, combined with studies performed on murine models [74], suggests that ION363 silences the mutant FUS transcript and results in a reduction of pathological FUS aggregates and a slowing down of motor neuron degeneration [74]. However, a single-patient study cannot determine whether ION363 modifies the course of the disease. The ongoing study NCT04768972
The increased number of the G4C2 repeats in the uncoded C9ORF72 region is the leading cause of fALS, responsible for 40% of fALS and 5–10% of sALS [76]. The expansion of G4C2 repeats in the C9ORF72 leads to specific processes such as haploinsufficiency, formation of RNA foci, and formation of dipeptide repeat proteins (DPR) [77]. Those changes are present many years ahead of the formation of TDP-43 aggregates in motor neurons, probably favouring the deposition of TDP-43 [78]. Researchers observed that DPR (polyGR) accelerates the formation of TDP-43 aggregation [77]. ASO designed selectively against V1 and V3 transcripts of C9ORF72 may lead to RNA foci and DPR reduction [78,79]. Administration of ASO on the C9ALS animal model showed a decrease of poly-GP and V3 transcript in cerebrospinal fluid [78,79]. Brown et al. were the first to apply ASO in treating C9ALS patients. The patients tolerated the therapy well, and polyGP reduction was observed [78].

The ATXN2 gene is mainly associated with sporocerebellar ataxia type 2 (SCA2), but an increased number of CUG (glutamine-encoding) repeats in ATXN2 was associated with a higher incidence of ALS [80, 81]. In addition, 4.7% of ALS patients have intermediate-length polyglutamine (polyQ) expansions in ataxin 2 [80]. Ataxin 2 is a protein involved in RNA metabolism, including stress granule assembly [82]. Intermediate-length PolyQ expansions stimulate the formation of TDP-43 aggregates in motoneurons [83]. It has been observed that lowering the concentration of ataxin 2 reduces the TDP-43 aggregation and improves survival and locomotor function in transgenic TDP-43 mice [82]. The therapy proposed by the authors is the first ASO therapy designed against a gene that is not the direct cause of neurodegenerative disease [82]. Due to crucial, physiological TDP-43 cellular functions, it is not feasible to develop an ASO therapy that directly modifies TDP-43. Therefore the primary group of ALS patients could benefit from ASO targeting ATXN2 [82, 84].

Stimulation of protein disaggregation

In ALS, both familial and sporadic, there are several abnormalities in protein synthesis, especially TDP-43. To begin with cytoplasmic mislocalization, through the deposition of hyperphosphorylated protein in the form of aggregations, and to end with cutting off C-terminal fragments, ultimately leading to toxic protein aggregation in the patient’s brain and spinal cord. Reversing protein aggregation could be a promising therapeutic strategy [85]. Thermal shock proteins (chaperones) control other proteins’ quality and perform protective functions for proteins against stress factors such as temperature, chemicals, or oxidative stress. One of them, Hsp-104 isolated from S. cerevisiae, exhibits disaggregation abilities concerning toxic protein deposits. Studies report that genetically modified Hsp-104 can dissolve TDP-43 aggregates but does not prevent their formation [86]. Naturally occurring in the human body Hsp-70 and Hsp-90 are stimulated by a transcription factor HSF1. It was discovered that a small molecule called acrimoclomol could stimulate HSF1[87]. Activating the HSF1 pathway reduces cell levels of TDP-43 deposits [88,89]. However, after promising results in preclinical trials, phase III tests questioned acrimoclomol efficiency as a form of ALS treatment [90]. Hsp-90, in cooperation with its co-protein Sti1 and possibly Hsp-70, can alter TDP-43 toxicity [91]. Another function of Hsp-70 is to regulate autophagy by stimulating the binding of lysosomes to damaged proteins [92]. The Hsp-110 collaborates with the Hsp-70 protein family that functions as part of the disaggregation pathway. A study by Nagy et al. indicated that Hsp-110 could increase the survival time in the ALS murine model [93]. Serine-rich chaperone protein (SRCP-1), a novel chaperone protein that prevents protein aggregation in the cell culture of Huntington’s disease, has shown controversial results in ALS models [88]. More studies and optimization are needed to evaluate its effectiveness [94]. HspA5 is a chaperone protein that binds directly to TDP-43. Recent studies indicate that up-regulation of HspA5 in ALS may increase motor neuron survival by inhibiting TDP-43 misfolding and subsequent toxicity. [90].

Single chain variable fragment (scFv) obtained by molecular methods from monoclonal antibodies could be a promising approach in the treatment of neurodegenerative diseases like Huntington’s disease, Parkinson’s disease, and ALS.
A study conducted by Tamaki et al. indicated that scFv interacts with TDP-43 directly and hastens the proteolytic degradation of its aggregations. Moreover, refolding abilities of Hsp-70 enhance the degradation of the scFv-TDP-43 complex [95]. Specific scFv can also enhance the polyubiquitin chains bound to TDP-43 and stimulate proteasomal and autophagy degradation pathways [95, 96].

Table 1 summarises presented ALS treatment strategies.

## Table 1. Summary of presented strategies and their research phase

<table>
<thead>
<tr>
<th>Treatment strategy</th>
<th>Proposed mechanism of action in correlation with TDP-43</th>
<th>Clinical results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riluzole</td>
<td>Riluzole inhibits the kinase CK1δ and thus prevents the formation of hyperphosphorylated TDP-43 aggregations.</td>
<td>Prolongs median survival time by 3 months, compared to placebo [91].</td>
</tr>
<tr>
<td>Edavarone</td>
<td>Edavarone modulates antioxidant cell response mediated by Nrf2 and thus reduces oxidative stress, leading to TDP-43 aggregations.</td>
<td>Slows disease progression by 33% measured by ALSFRS-R scale [91].</td>
</tr>
<tr>
<td>SINEs (Selective inhibitors of nuclear export)</td>
<td>SINEs are drugs that block XPO1 and cause the accumulation of not exported molecules inside the nucleus, including the TDP-43 protein.</td>
<td>Strategy in the preclinical phase.</td>
</tr>
<tr>
<td>PARP inhibitors (PARPi)</td>
<td>Decreasing the Poli ADP-ribosylation PARPi suppresses the formation of stress granules.</td>
<td>Strategy in the preclinical phase.</td>
</tr>
<tr>
<td>Rapamycin (mTOR kinase inhibitor)</td>
<td>Rapamycin inhibits mTOR kinase, enhances autophagocytosis, and, as a result, promotes the elimination of TDP-43 deposits.</td>
<td>Currently in II phase trial [92].</td>
</tr>
<tr>
<td>ASO targeting ATXN2</td>
<td>Antisense oligonucleotides modulate the expression of ATXN2, leading to ataxin 2 concentration lowering and, as a result, a decrease in the TDP-43 aggregation.</td>
<td>Strategy in the preclinical phase.</td>
</tr>
<tr>
<td>Arimocloclomol</td>
<td>Arimoclomol activates the HSF1 pathway, stimulates HSP-70 and HSP-90 to alter TDP-43 misfolding, and stabilises TDP-43 conformation.</td>
<td>Arimoclomol failed in the phase II/III trial (Clinicaltrials.gov identifier NCT03491462) [84].</td>
</tr>
</tbody>
</table>

PB-TURSO combines two compounds: phenylbutyrate (PB) and taurursodiol (TURSO). Compounds of PB-TURSO have moderating effects on endoplasmic reticulum stress (PB) and mitochondrial dysfunction (TURSO), both mechanisms known as potential pathogenic factors in ALS. The CENTAUR trial showed that introducing PB-TURSO therapy prolonged the patient’s median survival by 6.5 months compared with the placebo [99].

Masitinib is a selective oral tyrosine kinase inhibitor that targets the c-KIT receptor. Experiments on the ALS model showed masitinib’s ability to regulate microgliosis and neuroinflammation [100]. The study demonstrated that early introduction (before functional impairment) of masitinib could prolong a patient’s survival time by 2 years compared with a placebo [101].

Reldesemtiv is a fast skeletal muscle troponin activator (FSTA) that sensitizes the sarcomere to calcium, enhancing muscular power. This phenomenon may be helpful in ALS and other neuromuscular diseases leading to muscle weakness and fatigue [102]. Reldesemtiv was tested in a phase II trial in patients with ALS. The ALS Functional Rating Scale-Revised (ALSFRS-R) decrease noticed a statistically significant fall. Reldesemtiv was most beneficial for patients with faster disease progression [102].

Neurotrophic factor-secreting mesenchymal stromal cells (MSC-NTF, NurOwn) are bone marrow-derived mesenchymal stem cells. They were modified ex vivo to secrete neurotrophic factors such as Glial Cell Line Derived Neurotrophic Factor (GDNF) and Vascular Endothelial Growth Factor (VEGF) [103]. Unfortunately, phase 3 research on the use of MSC-NTF for ALS treatment had not reached a statistically significant response to treatment or functional improvement compared to placebo. Nevertheless, analysis of patients’ cerebrospinal fluid (CSF) revealed improvements in CSF biomarkers related to neuroinflammation and neurodegeneration after using MCS-NTF whereas the placebo remained the same [104].

A platform trial is clinical research with a single master protocol that evaluates numerous therapies sequentially across one or more groups of patients and permits potential inclusion or
exclusions of new therapies in the future. This model allows for faster development by evaluating many therapies simultaneously [105]. HEALEY ALS platform trial (NCT04297683) is the first for ALS patients [105]. Currently, the research includes five potential drugs: Zilucoplan, Verdiperstat, CNM-AU8, Pridopidine, and SLS-005 Trehalose. The estimated study completion date is on December 2023. This platform trial will undoubtedly accelerate the development of effective ALS therapy [Clinicaltrials.gov identifier NCT04297683].

Conclusions

Amyotrophic lateral sclerosis is a progressive, debilitating disorder that leads to a patient’s death. Despite much research, approved therapies only slightly prolong patients’ lives. Therefore, it is essential to understand the pathogenesis of ALS and use this knowledge to prepare new treatments. This review aims to demonstrate a new therapy approach and its possible correlation with TDP-43 proteinopathy. It is too early to choose the most promising strategy because most of the presented therapies are in the preclinical phase. It requires clinical phase trials to evaluate the safety and effectiveness of those treatments reliably. However, numerous clinical trials demonstrated that the early introduction (before functional impairment) of ALS therapies has the best response to treatment. Hopefully, further therapy development will enhance patients’ prognoses.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

References


