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# Esclerose Lateral Amiotrófica: uma revisão de tópicos específicos

*Amyotrophic Lateral Sclerosis: a Review on Specific Topics*

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**RESUMO**

A Esclerose Lateral Amiotrófica (ELA) é uma doença neuromuscular rapidamente progressiva. Como o seu diagnóstico pode ser difícil, o atraso diagnóstico pode comprometer o tratamento otimizado. O objetivo desta revisão da literatura é descrever a epidemiologia, as bases moleculares, métodos de diagnóstico atuais e em desenvolvimento e analisar tratamentos atuais e ensaios clínicos recentes.

Foi efetuada uma revisão narrativa da literatura, que consistiu em pesquisa bibliográfica nas bases de dados *PubMed, Embase, ScienceDirect, Cochrane Library, Wiley Online Library, ResearchGate, SpringerLink, MDPI* e *SciELO*, para identificar artigos publicados entre 2018 e 2023. Estudos sobre epidemiologia, bases moleculares, métodos de diagnóstico, tratamento e ensaios clínicos recentes relativos à ELA foram incluídos. Foram também consultados livros de texto de Neurologia.

A idade média de início é 51-69 anos. A incidência e a prevalência parecem estar a aumentar ligeiramente. Fatores de risco são frequentemente mencionados.

A etiologia é desconhecida. Os mecanismos propostos incluem o processamento de RNA, homeóstase proteica e disfunção do citoesqueleto neuronal.

Os métodos de diagnóstico incluem estudos eletrofisiológicos (obrigatórios) e podem englobar estudos adicionais: neuroimagiologia, ecografia neuromuscular e análises laboratoriais e genéticas.

Quatro principais tratamentos modificadores da doença constituem o pilar do tratamento farmacológico. Novos tratamentos farmacológicos estão a ser investigados.

A ELA coloca desafios a nível de diagnóstico e tratamento. É necessária mais pesquisa, essencialmente para descoberta de tratamentos farmacológicos mais efetivos.

**Palavras-chave:** esclerose lateral amiotrófica, epidemiologia, patologia molecular, diagnóstico, terapia farmacológica.

**ABSTRACT**

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neuromuscular disease. As its diagnosis can be difficult, diagnostic delay can compromise optimal management. The aim of this literature review is to describe epidemiology, molecular bases and current and under development diagnostic methods of ALS and to analyze current treatment and recent clinical trials.

A narrative literature review was made, consisting of bibliographical search on PubMed, Embase, ScienceDirect, Cochrane Library, Wiley Online Library, ResearchGate, SpringerLink, MDPI and SciELO, to identify articles published between 2018 and 2023. Studies concerning epidemiology, molecular bases, diagnostic methods, treatment, and recent clinical trials of ALS were included. Neurology textbooks were also consulted.

Mean age of onset is 51-69 years. Incidence and prevalence seem to be slightly increasing. Risk factors are frequently mentioned.

Etiology is unknown. Proposed mechanisms include RNA processing, protein homeostasis and neuronal cytoskeleton dysfunction.

Diagnostic methods imply electrophysiological studies (mandatory) and may comprise additional studies: neuroimaging, neuromuscular ultrasound, laboratory testing and genetic testing.

Four main disease-modifying treatments are mainstay of pharmacological treatment. New pharmacological treatments are under investigation.

ALS poses diagnostic and treatment challenges. Further research is necessary essentially to discover more effective pharmacological treatment.

**Keywords:** amyotrophic lateral sclerosis, epidemiology, molecular pathology, diagnosis, pharmacological therapy.

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## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most frequent adult motor neuron disease (MND). MND affect the voluntary motor system including anterior horn cells, certain motor cranial nerve nuclei and corticospinal/corticobulbar tracts[1,2].

ALS is a rapidly progressive neuromuscular disease that destroys upper motor neurons (UMN) and lower motor neurons (LMN). It can manifest symptoms and signs of UMN dysfunction (weakness, spasticity, hyperreflexia, pathological reflexes, pseudobulbar affect) and LMN dysfunction (muscle weakness, atrophy, or cramping, fasciculations, hypotonia, hyporeflexia)[1,2,3].

ALS originates skeletal muscle weakness, eventually leading to need for ventilatory support or death from respiratory failure. The onset of weakness may be in limbs, bulbar muscles, or respiratory muscles[1,2]. Focal onset is most common[3]. ALS may also encompass skeletal muscle dysfunction[4], fatigue[5] and cognitive, behavioral and/or psychiatric abnormalities[1,3,6].

Characteristically, patients seek evaluation complaining of focal weakness and atrophy[1,2].

Diagnosis of ALS is suggested by manifestations of UMN and LMN dysfunction[1,2].

Because of diverse factors (related to patients, clinicians, and healthcare system), the diagnosis of ALS can be difficult, resulting in diagnostic delay that can compromise optimal management[7-10]. Diagnostic delay is longer in limb-onset ALS[7].

Artificial intelligence and statistical methods can help to identify, stratify, and predict ALS progression, but its application is difficult[8,11,12].

The majority of ALS is sporadic; approximately 10% is familial and usually autosomal dominant[1].

This paper is a literature review about ALS, particularly the following topics: Epidemiology; Molecular bases; Current and under development diagnostic methods; Analysis of current treatment and recent clinical trials.

## EPIDEMIOLOGY

Mean age of ALS onset is 51-69 years[13-22].

European patients normally have a later age at onset[13,14]. Patients with familial ALS have an earlier age of onset than patients with sporadic ALS[13,14].

Incidence of ALS is 0.6-3.8 per 100000 person-years (PY)[13-27]. In Europe, incidence is higher (2.1-3.8/100000 PY)[13,14,16,17,21]. There are reports of incidences of 3.8/100000 PY (Stockholm 2014 and Scotland 2015-2017[13,21]), 2.1/100000 PY (Norway 2000-2015[17]), 2.8/100000 PY (2002-2014 Italy[16]) and 2.71/100000 PY (2018-2021 Italy[15]).

Incidence seems to be increasing[13,14,17,19,21] (for instance, during a 25-year period in Scotland, there was an increase of 36%[21]), although probably due to people living longer and better recognition of diagnosis[21].

Male-to-female incidence ratio is between 1 and 2[13-21,28,29].

Prevalence is 4.1-8.4/100000 persons[13-19,21,22,24-26,28,29] and seems to be slightly increasing[13,14,18,21,22,28,29].

There is significant heterogeneity in reported incidence and prevalence within and between countries/geographic regions[13,25,30].

There is a projected increase in ALS

in 2015-2040, mainly due to aging of population, particularly in developing countries[31].

Sporadic ALS probably involves genetic susceptibility to environmental risk factors. Established risk factors include older age, male sex, and family history[13,14,26].

Environmental risk factors need further research. The more common are head trauma, electrical burns, military service, pesticides, solvents, exposure to heavy metals, heavy labor, lower socioeconomic status occupations, alcohol intake, viruses, magnetic field, dietary habits, high body mass index (BMI), nutritional state and physical activity[26,32-40]. The more consistent are head trauma, electrical burns, pesticides and exposure to heavy metals. Studies report conflicting results, especially regarding physical activity. In some, physical activity seems to be a risk factor[33,34], while in others there is no clear evidence that it influences incidence[38,41]. High-quality population-based studies or disease registers are necessary from outside Europe to better estimate disease burden and understand risk factors[13,14,24].

Survival of ALS is variable[13]. Mean survival time from symptoms onset to death or invasive respiratory support is 24-50 months[13,17,18,42].

Predictors of longer survival comprise male sex, longer diagnostic delay, attending multidisciplinary clinics, spinal onset, younger age at onset and diagnosis, higher baseline ALSFRS-R (ALS Functional Rating Scale-Revised) score, higher BMI, and weight gain after diagnosis[13,17,19,21,43-45].

In Europe, creation of population-based registries was fundamental. The first registry was established in Scotland in 1989, followed by England, Ireland, and Italy, aiming fundamentally at studying clinical features of MND in populations. They measured an incidence of 1.7-2.3/100000 PY, much higher than reported previously, due to improvement in data collection methods. In 2004, EURALS was created – a consortium of all European population-based registries. Since then, ALS epidemiology in Europe improved[30,46].

In EURALS, the estimated incidence of ALS in Europe was 2.2/100000 PY[30]. Incidence had a peak in the seventies, decreased rapidly after 80 years of age and was homogeneous across different countries[46]. Geographic differences in incidence are partly due to both genes and environmental risk factors[30]. Survival was around 25 months in Northern Europe and around 30 months in Western/Southern Europe[30]. Some studies suggest that incidence depends on latitude gradient (higher in North and lower in South[15,30,42]).

The known gene more often associated with ALS is *C9ORF72*, responsible for about 40% of familial cases and 8% of sporadic, and particularly highly prevalent in Finland[30] (which has one of the highest prevalence and incidences worldwide[30,42], with differences between east and southwest[42]). Viking migration was the prime responsible for spreading *C9ORF72* in Europe and North America, suggesting that part of geographic distribution may be of genetic origin[30].

## MOLECULAR BASES

Etiology of ALS remains unknown[26,47,48], however, several mechanisms and associated genes were described. One me-

chanism is related to RNA processing and involves many RNA-binding proteins (RBP).

Trans-active response DNA-binding protein (TDP-43), a protein encoded by *TARDBP* gene, is implicated in RNA splicing, transport, stability, and suppression and was the first RBP to be associated with ALS[48-50]. Mutations in *TARDBP* are present in 1-5% of sporadic cases[49,51] and 3-5% of familial cases[49,51,52]. To perform its function, TDP-43 contains in its structure a region called low-complexity domain (LCD)[48], essential for interaction with RNA and other proteins. While necessary for its function, it also makes TDP-43 prone to aggregation[48]. (In other literature, it´s considered a prion-like domain[52]). LCDs, together with a mechanism called liquid-liquid phase separation (LLPS), allow formation of granules containing RNA and RBPs in cytoplasm of neurons[48]. An example is stress granules, created in times of cellular stress, that sort mRNAs in a triage process that determines their fate (translation, sequestration or degradation)[51,52]. Mutations in LCD[26,48] and mislocalization in cytoplasm (TDP-43 is predominantly nuclear[52]) lead to accumulation of cytoplasmic TDP-43 and its continuous aggregation to form cytoplasmic inclusions that are a hallmark of ALS48. These inclusions are seen in 90-97% of ALS cases[48,53]. Although they play an uncertain role in pathogenesis, their frequency and the fact that reduction of these aggregates is neuroprotective means they have a potentially important role[26,48]; dysregulation of TDP-43 protein turnover, impairment of endocytosis, mitochondrial dysfunction induced by TDP-43 localization, dysregulation of metal ion homeostasis and interference with chromatin remodeling have been proposed as mechanisms[54].

Fused in sarcoma (FUS) is another RBP, involved in transcriptional regulation, splicing, subcellular localization and translation[48]. Mutations in FUS occur in 4% of familial cases and 1% of sporadic cases[51]. Pathogenic mechanism is very similar to that of TDP-43, including presence of a prion-like LCD, cytoplasmic mislocalization, inclusion in granules and aggregation[48,51,52].

A different mechanism is related to protein homeostasis. Mammalian cells have two maintenance mechanisms to degrade defective, damaged or unnecessary proteins: ubiquitin-proteasome system (UPS) and autophagy[48]. In UPS, a protein is tagged for degradation through attachment to ubiquitin by three enzymes: E1, E2 and E3; it is then unfolded and cleaved by passage through the proteasome, a protein complex containing proteases that break down tagged proteins into smaller peptides[48]. In autophagy, protein aggregates are engulfed into double-membrane structures called autophagosomes, which then fuse with lysosomes for degradation[48]. In ALS these systems are disturbed because of mutations in genes that encode proteins necessary to these systems (eg. ubiquilin 2, sequestosome 1 and valosin-containing protein)[51,52] and also because of overwhelming due to excess TDP-43[48].

The first gene associated with ALS was SOD1. It encodes superoxide dismutase 1 enzyme (SOD1), which dismutes superoxide radicals into hydrogen peroxide, a less oxidizing species, protecting the cell from oxidative damage[49-51].

SOD1 mutations are found in 12-20% of familial[49-51] and 0,7-4% of sporadic cases[26]. Although loss of function leads to oxidative stress that causes cell death, pathogenesis results instead from aggregation caused by misfolding due to mutations and post-translational modifications[26,48-52]. As with TDP-43, overwhelming of UPS and autophagy systems occurs[51]. Interestingly, study of SOD1 pathogenesis highlighted the role of non-neural cells in ALS: in studies using transgenic mice, expression of mutant SOD1 in neurons didn't lead to disease, whereas expression of mutant SOD1 in neighboring cells induced motor neuron degeneration[26,52].

Another common genetic cause is expansion of a G4C2 hexanucleotide sequence in *C9ORF72* gene. Located in the non-coding region of that gene, these repeats, normally never exceeding 30 copies, may expand up to hundreds or thousands of copies[51,52,55]. Three possible pathogenic mechanisms were proposed: transcription of hexanucleotide repeat expansion (HRE) generates toxic RNA that binds and sequesters multiple RBPs, resulting in dysregulation of RNA processing[51,55-57]; these RNAs may be translated even without a start codon, resulting in dipeptide repeat proteins that are toxic and aggregation prone, forming intracellular inclusions [26,51,55,57]; and presence of HRE may lead to reduced *C9ORF72* expression through epigenetic mechanisms[26,51,56,57] (*C9ORF72* is a protein involved in autophagy, vesicular trafficking and other functions[57]), although it isn´t known if this is pathogenic or protective[51,57].

Another proposed mechanism regards the neuronal cytoskeleton. Motor neuron axons can be a meter long; such a size requires a functional cytoskeleton structure for cell shape and axonal transport of organelles and vesicles to and from synapses[26,51,52]. In ALS, dysfunction may occur in cytoskeleton structure and in its transport function. Neurofilaments (NF) are part of cytoskeleton structure; NF heavy chain and peripherin are two NF components detected in aggregates of ALS patient's motor neurons, and mutations in their respective genes are associated with ALS[51,52,58,59]. The dynactin complex connects cargo to motor protein dynein and is essential to dynein's movement along the microtubules; mutations in dynactin subunit 1 gene *(DCTN1)* have been associated with familial and sporadic cases, through disturbance of intracellular transport that impairs neuronal growth and synapse maturation[26,58,59].

## DIAGNOSTIC METHODS

Diagnosis of ALS is suggested by manifestations of UMN and LMN dysfunction[1,2].

Diagnostic criteria of ALS were created and suffered alterations over time. During several years, El Escorial revisited criteria were used to ascertain diagnosis of ALS once other diseases were excluded[60]. In 2006, the Awaji criteria modified the revised El Escorial criteria to further integrate electrophysiological criteria with clinical examination findings, and to add the presence of fasciculations as a LMN sign that could substitute for fibrillation potentials-positive sharp waves in muscles with neurogenic changes[61]. Though, both criteria were complex and lacked sensitivity.

In 2019, new diagnostic criteria for ALS

emerged (Gold Coast criteria): progressive motor impairment documented by history or repeated clinical assessment, preceded by normal motor function; and presence of UMN and LMN dysfunction in at least 1 body region or LMN dysfunction in at least 2 body regions; and investigations excluding other disease processes[3]. Gold Coast Criteria have higher sensitivity and similar specificity than previous ALS criteria.

Diagnostic evaluation of ALS implies electrophysiological studies (mandatory) and may comprise additional studies: neuroimaging, neuromuscular ultrasound, muscle biopsy, laboratory testing and genetic testing.

Electrophysiological studies - nerve conduction studies and electromyography (EMG) - are central in establishing diagnosis of ALS and investigating other possible diagnoses.

Electromyography is the standard neurophysiological study to detect LMN dysfunction in ALS. It is essential to exclude pathologies that mimic ALS and to achieve early diagnosis[62].

Electrodiagnostic characteristics of ALS include normal sensory nerve conduction studies, normal or low motor amplitudes depending on disease stage, and normal distal motor latencies and conduction velocities. With loss of motor amplitudes, conduction velocities may diminish. EMG generally demonstrates decreased recruitment pattern (which signals loss of motor units), large and/or complex motor unit action potentials, and abnormal spontaneous activity (positive sharp waves, fibrillation potentials, fasciculations and complex repetitive discharges)[2].

Currently, needle electromyography (nEMG) is the EMG method usually used for electrodiagnosis of ALS. Surface electromyography (sEMG) is becoming a more practical and less painful alternative to nEMG but still has analytical and technical challenges[63].

Motor unit estimates are useful to quantify LMN loss. Electrical impedance myography is emerging as a way to monitor ALS progression. Neurophysiological investigation of UMN dysfunction is difficult; detecting decreased cortical inhibition by threshold tracking cortical magnetic stimulation seems a promising method[62].

Repetitive nerve stimulation[64], single-fiber EMG[64] and transcranial magnetic stimulation[62,64,65] may be performed in selected patients to improve diagnostic testing and help identify or exclude alternative diagnoses.

The preferred modality of neuroimaging is magnetic resonance imaging (MRI) of brain and spine. Specific imaging techniques to detect UMN disease in ALS include magnetic resonance spectroscopy and diffusion tensor imaging[64,66-69].

Positron emission tomography (PET) imaging with specialized tracers indicating glial activation is also a possible diagnostic aid[70].

Neuromuscular ultrasound may be very useful when electrophysiological studies remain inconclusive[71-74]. As fasciculations are readily detected by ultrasound, it´s being increasingly used to diagnose ALS, combined with EMG (combination augments diagnostic accuracy)[62].

Muscle biopsy isn´t a routine diagnostic study but may be performed if myopathy is suspected[64].

In terms of laboratory methods, biomarkers exist for ALS and investigation

is ongoing, due to need to provide accurate diagnosis, reduce delay from symptom onset to diagnosis and for prognosis[75,76,77].

Neurofilaments, as stated above, are components of the neuronal cytoskeleton. They´re distinguished by molecular weight into light (NFL), medium (NFM) and heavy (NFH) chains[75]. NFL and phosphorylated NFH (pNFH) are examinable in serum and cerebrospinal fluid (CSF) and its presence is considered an indicator of ongoing neuronal damage[70,75]. Many studies have correlated serum and CSF levels of NFL and pNFH with ALS, being higher than healthy subjects[70,75,78-80]; high levels are also associated to more aggressive disease progression and shorter survival[75,81]. NF have the disadvantage of being unspecific because they´re detected in other neurodegenerative diseases (Alzheimer's disease, Huntington's disease, frontotemporal dementia)[82,83]. NF concentration is very low in CSF (it is measured in picograms per milliliter, pg/mL) and is even lower in serum, requiring very sensitive analytical techniques[75,79,82]. Initially, the technique used was enzyme-linked immunosorbent assay (ELISA), however, it isn´t sensitive enough for concentrations found in blood[75,79] and it requires collecting CSF through lumbar puncture[75,79,81]. Methods recently developed, based on electrochemiluminescence immunoassay and single molecule array (Simoa), which are more sensitive, allow detection of NF in blood samples, which are much more simple and easier to obtain[75,79,81,56].

Simoa is similar to ELISA, using a sandwich antibody complex formed on microscopic beads; however, in case of very low antigen concentration, in ELISA the signal (fluorescence or other) is diluted in the assay volume and is too low to be detected. In Simoa, the beads containing the immunocomplexes are loaded into thousands of microwells, each fitting only one bead. After sealing, signal is detected in each well using microscope optics. Wells with a bead containing immunocomplex are "on", whereas wells with a bead not containing immunocomplex are "off". The number of "on" wells relative to the "off" wells determines the analyte concentration. Detecting single "on" beads enables measurement of analytes at extremely low concentrations (in the femtomolar range)[84].

ALS has an inflammatory component, with inflammation occurring in central nervous system and being detected in blood[70,83]. Inflammatory biomarkers have been studied to evaluate correlation with disease stage, progression rate and risk of death[70,83,85]. High levels of the most studied inflammatory marker, C-reactive protein (CRP), were associated with increased risk of death, although the association wasn't constant[85]; it was also considered to have a weak correlation with disease duration[83]. Many other biomarkers, such as immune cell populations and cytokines, were studied, but findings were heterogeneous[70,83].

Creatine kinase (CK) is a muscle enzyme; high levels in blood indicate muscle damage, so CK could be a biomarker for a disease that manifests as muscle atrophy[86]. CK levels in blood are found to be higher in ALS patients than healthy controls[86,87], although not in all cases[87,77]. Levels are higher in cases of LMN loss that leads to muscle atrophy[86,87]. Simi-

larly, they were higher in cases with limb-onset[77]. Correlation with survival was conflicting, with some studies correlating with longer survival[70,86] and others not finding relation[77,87].

Creatinine, a metabolite of creatine[77,85] and usually used as biomarker of renal function[87], was also studied as a biomarker of ALS. Low serum creatinine was associated with disease progression[87] and decreased survival[85,87], as well as increased disease severity at diagnosis[77]. Furthermore, it was reported that creatinine levels start decreasing 2 years before diagnosis[87], with another study reporting a slow decrease before symptom onset and diagnosis and accelerated decrease afterwards[88].

As stated above, mutations in many genes have been associated with ALS. Although genetic testing isn´t used for diagnostic purposes, it has implications in terms of exclusion of genetic disorders like ALS, identification of patients eligible for trials of treatments targeting specific mutated genes, counseling of family members for predictive testing and prognosis[70]. Recent guidelines recommend that all ALS patients should be offered genetic testing that includes *C9ORF72*, SOD1, FUS and TARDBP[89].

## CURRENT TREATMENT AND RECENT CLINICAL TRIALS

ALS is, at present, incurable[26,64]. Current treatment options focus on disease-modifying therapies and maintenance of quality of life[90]. The oldest approved disease-modifying treatment is riluzole, approved in the United States (US) in 1995 and in the European Union in 1996[91]. Its mechanism of action, not fully understood, is thought to result from different actions centered in glutamate excitotoxicity[91,92]. These actions include sodium channel blocking on presynaptic neurons, which decreases glutamate release into the synaptic cleft[92]; increased glutamate reuptake by astrocytes[92]; noncompetitive antagonism of glutamate receptors[92]. Excess glutamate leads to an excess of calcium ions entering the cell, causing damage to endoplasmic reticulum, nucleic acids, and mitochondria, resulting in neuronal death[26,91]. Riluzole was found, in first clinical trials, to increase survival by two to three months[91,92]; subsequent studies and real-world evidence revealed an even greater survival benefit, ranging from 6 to 21 months[91,93]. Riluzole is available as 50 mg tablets, taken orally twice daily[91,92].

It took 20 years for a new drug to be available. Edaravone was approved in 2015 in Japan and South Korea, 2017 in the US, 2019 in China and Switzerland, 2020 in Indonesia and Malaysia and Thailand in 2021[92]. At the time of writing, edaravone is not approved in the European Union. Edaravone is an antioxidant which acts by scavenging reactive oxygen species responsible for oxidative stress and cell death[90]. Its exact mechanism in ALS is unknown[93]. There is controversy on the efficacy of edaravone, with some trials reporting (in groups with characteristics of early disease) an improvement in ALSFRS-R score and better scoring in ALSAQ-40 questionnaire that evaluates quality of life, whereas other trials didn't show any efficacy[90-93]. Edaravone is administered intravenously, 60 mg/day, for 14 days, followed by 14 days drug-free (first cycle, subsequent cycles are 10 of 14 days

with drug, then 14 days without)[91-93]. In 2022, a more convenient oral suspension of 105 mg/day was approved in the US[92].

A combination of taurursodiol and sodium phenylbutyrate (PB-TURSO) was approved in the US and in Canada in 2022[94]. Both substances were previously approved for other uses – taurursodiol for chronic cholestatic liver disorders and sodium phenylbutyrate for hyperammonemia due to urea-cycle disorders[92]. The combination of both inhibits neuronal apoptosis by ameliorating toxicity from endoplasmic reticulum (sodium phenylbutyrate) and decreasing mitochondrial dysfunction (taurursodiol)[92,95]. PB-TURSO was reported, after a phase II trial with 137 patients, to prolong survival by 6,5 months and slow down disease progression (measured by the ALSFRS-R)[95]. A larger phase III trial, enrolling 600 patients, is ongoing[96]. Another ongoing phase III trial is testing taurursodiol as add-on to riluzole (taurursodiol and riluzole vs riluzole alone)[94]. PB-TURSO is available as powder for oral suspension containing 3 g of sodium phenylbutyrate and 1 g of taurursodiol[92].

Tofersen is an antisense oligonucleotide (ASO). It binds to SOD1 mRNA, inducing its degradation and decreasing production of SOD1 protein[92-94,97]. It has been investigated in patients carrying SOD1 mutations[92,97]. In a phase I/II trial with 50 patients with SOD1 mutations, to whom tofersen was administered intrathecally, SOD1 protein in CSF and pNFH and NFL in CSF and plasma had decreased, in the group that had received the 100 mg dosage. Other data suggested that tofersen could slow functional decline, assessed by ALSFRS-R[94]. Based on this report, a larger, phase III trial enrolling 108 patients was performed; this trial failed to reveal any difference in ALSFRS-R decline between a group taking 100 mg of tofersen and a control group taking placebo after 28 weeks[92,94,98]. Biomarker (NFL and SOD1) findings were like the previous trial[92,98]. In the subsequent open-label long-term trial, it was revealed that the patients that had started treatment earlier had a smaller decline in ALSFRS-R than patients that started in the open-label phase[92,94,98]. The long-term trial is still ongoing[92]. Another ongoing trial with tofersen is evaluating its effect in early intervention on presymptomatic carriers of SOD1 mutations[92,94,98]. Tofersen received accelerated approval in the US in April 2023[99].

At the time of writing, these are the only four disease-modifying treatments approved anywhere in the world for ALS. Many more are in experimental phases, ranging from small molecules to antibodies to genetic and stem cell therapies. Some of the most promising and/or in more advanced stages of investigation will be described.

Masitinib is a tyrosine kinase inhibitor that reduces neuroinflammation by acting in the microglia, macrophages, and mast cells in the central and peripheral nervous systems, thus exerting a neuroprotective effect[92,93,98]. It is administered orally. It was tested in a phase II/III clinical trial with 394 patients, in association with riluzole, during a period of 48 weeks. It resulted in a 27% slowing of ALSFRS-R deterioration, with positive results also in deterioration of quality of life (measured by ALSAQ-40) and of

respiratory function. Long-term overall survival was also increased by two years with 4,5 mg/kg/day of masitinib[92,93,98]. A phase III clinical trial, with an enrollment of 495 patients, testing masitinib in association with riluzole, is ongoing[93,98].

ION363 (jacifusen) is another ASO (same technology and mechanism as tofersen), aimed at FUS mRNA. It is under study in a phase III trial[92,98].

Ibudilast is an orally administered small molecule that inhibits macrophage migration, phosphodiesterases and proinflammatory cytokines and promotes neurotrophic factors and anti-inflammatory cytokines; all this attenuates glial cells, with neuroprotective effects[92,97,98,100]. It also increases autophagosomal clearance of SOD1 and TDP-43 aggregates[92,97,98]. A phase IIb/III trial, including 230 patients, is testing ibudilast's efficacy and safety in association with riluzole for 12 months[97,98,100]. A previous phase I/II trial showed no overall difference in disease progression[97].

Fasudil is a Rho kinase (ROCK) inhibitor, administered intravenously. It's expected to be neuroprotective by counteracting neural apoptosis, promoting axonal regeneration, and regulating microglia[98,100]. It's currently being tested in Europe in a phase II trial with 120 patients[92,98,100].

Methylcobalamin is a form of vitamin B12. At a very high dosage, it's reported to protect from glutamate neurotoxicity and induce nerve regeneration[97,98]. A phase II/III testing 25 mg and 50 mg of methylcobalamin against placebo found no difference in survival or ALSFRS-R; post-hoc analyses showed prolonged survival and delayed progression in the specific group of patients of the 50 mg group who had been diagnosed 12 months or less prior to the study[97,101]. A posterior phase III trial, with 130 patients diagnosed within one year before the study, revealed reduced clinical deterioration in the group treated twice weekly with 50 mg intramuscular methylcobalamin for 16 weeks[92,97,98].

NurOwn is a cell treatment consisting of mesenchymal stem cells (MSC), collected from the patient's own bone marrow, manipulated to produce neurotrophic factors (NTF) and transplanted intrathecally back into the patient[94,101]. After positive results in previous trials, a phase III trial showed no improvement in disease progression (measured by ALSFRS-R), but improvements were seen in CSF biomarkers of neuroinflammation, neurodegeneration and NTFs[92,94]. Lenzumestrocel is another treatment consisting of bone marrow MSCs. It's currently under evaluation in a phase III trial[92,94].

Aside from new drugs and treatment strategies, repurposing of existing drugs is also under study. Memantine is a drug used in Alzheimer's disease that acts as a noncompetitive antagonist of the N-methyl-D-aspartate receptor, a glutamate receptor, so it may contribute to ALS treatment by acting on glutamate excitotoxicity[92,98]. It was under evaluation in a multiarm, phase II/III trial along with trazodone, an antidepressant that inhibits protein kinase RNA-like endoplasmic reticulum kinase (PERK) and reduces the formation of stress granules[92,98]; each recruiting arm is assigned only one treatment (trazodone or memantine) or placebo[102]. Both drugs were removed from the trial due to lack

of benefit; the trial is currently only testing amantadine, a drug currently used for Parkinson's disease and similar in structure to memantine[102].
Pharmacological treatment can also be symptomatic (e.g., anti-spastic medications for spasticity, analgesics for pain, antidepressants for depression…).
Supportive and palliative care are also important forms of non-pharmacological treatment in patients with ALS. Physical and rehabilitation treatments, including physical, occupational and speech therapy can help to preserve patients' autonomy and prevent rapid functional decline[92,103-109]. Access to palliative care can improve quality of life of both patients and caregivers[110-112].

## CONCLUSIONS

ALS is the most frequent adult motor neuron disease. It is rapidly progressive and fatal. It destroys both upper and lower motor neurons. Therefore, its diagnosis is suggested by manifestations of UMN and LMN dysfunction.
Because of diverse factors, its diagnosis can be difficult, resulting in diagnostic delay that can compromise optimal management.
Artificial intelligence and statistical methods can help to identify, stratify, and predict ALS progression, but its application is difficult.
Mean age of ALS onset is 51-69 years. Men are more affected than women. Incidence and prevalence seem to be slightly increasing. There is relevant heterogeneity in reported incidence and prevalence within and between countries/geographic regions.
About 90% of ALS is sporadic and 10% is familial. Sporadic ALS probably involves genetic susceptibility to environmental risk factors. Several environmental risk factors were identified, but need further research. Established risk factors include older age, male sex, and family history. The known gene more often associated with ALS is *C9ORF72*.
In Europe, creation of population-based registries was fundamental for improving ALS epidemiology.
Even though some mechanisms have been proposed for the pathogenesis of ALS, the etiology of most cases remains unknown. The proposed mechanisms explain a considerable part of the familial cases of ALS, but only a small minority of the sporadic cases. Nevertheless, some features of these mechanisms are a hallmark of ALS, such as cytoplasmic inclusions of protein aggregates. This aggregation is the result of dysfunction in RNA processing and protein homeostasis. Neuronal cytoskeleton dysfunction is another proposed mechanism.
Diagnostic criteria of ALS changed over time. Diagnostic evaluation of ALS implies obligatorily electrophysiological studies and may comprise additional studies (neuroimaging, neuromuscular ultrasound, laboratory testing and genetic testing). Electrophysiological studies (nerve conduction studies and EMG) are central in establishing diagnosis of ALS and investigating other possible diagnoses.
At the time of writing only four disease-modifying treatments are approved, with limited effect in survival and disease progression, and only one of them is available worldwide. Riluzole has been the standard of treatment for almost 30 years – and the only treatment for most of that time. More recen-

tly, edaravone, PB-TURSO and tofersen were approved, but only in a few countries. Investigation of new treatments is ongoing, on technologies including small molecules, antibodies, genetic treatments and stem-cell therapies.
Other treatment modalities include symptom-oriented pharmacological treatment and supportive and palliative care.

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