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## Abstract

Although the past two decades have produced exciting discoveries in the genetics and pathology of amyotrophic lateral sclerosis (ALS), progress in developing an effective therapy remains slow. This review summarizes the critical discoveries and outlines the advances in disease characterization, diagnosis, imaging, and biomarkers, along with the current status of approaches to ALS care and treatment. Additional knowledge of the factors driving disease progression and heterogeneity will hopefully soon transform the care for patients with ALS into an individualized, multi-prong approach able to prevent disease progression sufficiently to allow for a dignified life with limited disability.

## Introduction

As the most common cause of adult onset motor neuron disease (MND), amyotrophic lateral sclerosis (ALS) is traditionally classified as a neuromuscular disorder because the presenting symptoms are caused by muscle weakness and atrophy. However, clinical, genetic, and molecular discoveries over the past 20 years have challenged this convention. ALS shares features of frontotemporal dementia, a group of neurodegenerative disorders that causes cognitive, behavioral, and motor dysfunction. Nearly half of all patients with ALS have varying degrees of cognitive and/or behavioral impairment, with approximately 15% meeting the diagnostic criteria for frontotemporal dementia.[1] Conversely, about 15% of patients with behavioral variant frontotemporal dementia and 18% of patients with primary progressive aphasia have ALS.[2 3] These disorders also have overlapping genetics, with hexanucleotide repeat expansion (HRE) in *C9ORF72* being the most common genetic cause of ALS, frontotemporal dementia, or both in people of European ancestry.[4] Additionally, abnormal aggregation of transactive response DNA binding protein 43 (TDP-43) or fused in sarcoma (FUS) is present in the cytoplasm of cortical neurons in ALS and frontotemporal dementia.[5 6] As such, ALS is widely recognized as a complex neurodegenerative disorder in the frontotemporal dementia-MND continuum.[7 8] The reconceptualization of ALS in this continuum of disorders has allowed for novel approaches toward understanding fundamental disease mechanisms contributing to pathogenesis and has opened new avenues in approaches toward therapy. In this review, we provide a comprehensive summary of the clinical and genetic heterogeneity of ALS and advances in molecular pathology and biomarkers, and we highlight key interventions that improve quality of life. The intended audience includes students, trainees, general neurologists, and neuromuscular subspecialists.

## Epidemiology

The worldwide prevalence and incidence of ALS are estimated to be 4.42 per 100 000 population and 1.59 per 100 000 person years, respectively, and population based studies have shown geographic variation with the highest in western Europe (prevalence 9.62 per 100 000 population and incidence 2.76 per 100 000 person years) and lowest in South Asia (prevalence 1.57 per 100 000 population and incidence 0.42 per 100 000 person years). The incidence and prevalence of ALS are higher in developed regions, and a temporal trend has been observed, with the incidence rising by 0.00013 per year.[9 10] The prevalence and incidence of ALS is higher in men (prevalence 5.96 per 100 000 population; incidence 1.91 per 100 000 person years) than in women (prevalence 3.90 per 100 000 population; incidence 1.36 per 100 000 person years).[9 10]

## Sources and selection criteria

We independently did searches using the Boolean search criteria in the PubMed and Embase databases, between January 1990 and December 2022, using search terms such as amyotrophic lateral sclerosis, motor neuron disease, frontotemporal dementia, diagnosis, diagnostic criteria, prognosis, genetics, pathology, biomarker, and treatment. We identified articles published in the English language and selected them for inclusion on the basis of other criteria including relevance, peered review, and study type (randomized controlled trials, systematic reviews and meta-analyses, and observational studies). We prioritized publications in high impact

and ALS specific journals published in the past 15 years. Several important publications could not be included owing to the scope of this review. We excluded case reports and articles not published in English.

## Clinical complexity of ALS

### Clinical heterogeneity

ALS is a clinically heterogeneous disorder (fig 1), and the biological underpinning of the heterogeneity is poorly understood. Typically, the symptom onset is localized with spread of motor impairment to adjacent muscle groups and/or regions of the neuroaxis. Usually, the progression rate is linear for any given person, but the rate often varies between patients.[11] ALS of spinal onset with weakness first appearing in limb muscles occurs most frequently (two thirds of patients), followed by bulbar onset with initial weakness in lingual and oropharyngeal muscles (a third of patients). Axial or respiratory muscles are rarely the first to be affected.[12] Uncommon subtypes of ALS with spinal or limb onset exist, with atypical patterns of weakness in which the motor impairment tends to be regionally confined early in the disease course. In brachial amyotrophic diplegia or flail arm syndrome, weakness tends to affect proximal upper extremities symmetrically. Similarly, in flail leg syndrome or lower extremity amyotrophic diplegia, weakness is mainly in the lower extremities.[13] Other rarer ALS phenotypic variants include isolated bulbar ALS and hemiplegic ALS presenting with asymmetric hemibody weakness.[14] Although the symptoms rarely remain restricted in these subtypes, the progression is typically slow.[12]

Another contributor to phenotypic heterogeneity is the burden of neuronal degeneration in the cortex (upper motor neuron; UMN), brainstem, and spinal cord (lower motor neuron; LMN). Typically, the neurological examination shows UMN and LMN dysfunction, but the contribution of each likely falls on a continuum and can vary with patients having predominantly upper or lower motor signs (fig 1).[11] One explanation for this variability may be differences in the pattern of spread in the course of the disease.[11 15] At the extremes are rare phenotypes such as primary lateral sclerosis (PLS) presenting as a pure UMN disorder and progressive muscular atrophy (PMA) presenting as a pure LMN disorder.[16-18] Some clinical features distinguish PLS from typical ALS. In PLS, symptoms are symmetric and slowly progressive, and they frequently have an ascending pattern of spread.[19] PMA is clinically similar to typical ALS in the rate and pattern of symptom spread, but a subgroup of PMA may have slower disease progression.[17] Prognostic factors associated with longer survival include UMN or LMN predominant symptoms, flail arm variant, and younger age at onset. Factors associated with shorter survival include bulbar and/or respiratory onset, comorbid frontotemporal dementia, poor nutritional status, neck flexion weakness, and older age at onset.[12]

### Cognitive and behavioral dysfunction

Although ALS is synonymous with MND, cognitive and/or behavioral dysfunction are recognized core clinical features.[1] Neuropsychological abnormality is associated with faster disease progression and shorter survival and occurs more frequently in advanced disease.[20] Motor symptoms that alert patients and their care givers to a neurological disorder may overshadow antecedent or concurrent neuropsychological symptoms.[21] Because cognitive or behavioral changes may be obscured by motor dysfunction, validated screening tests specific for ALS such as the Edinburgh Cognitive and Behavioral

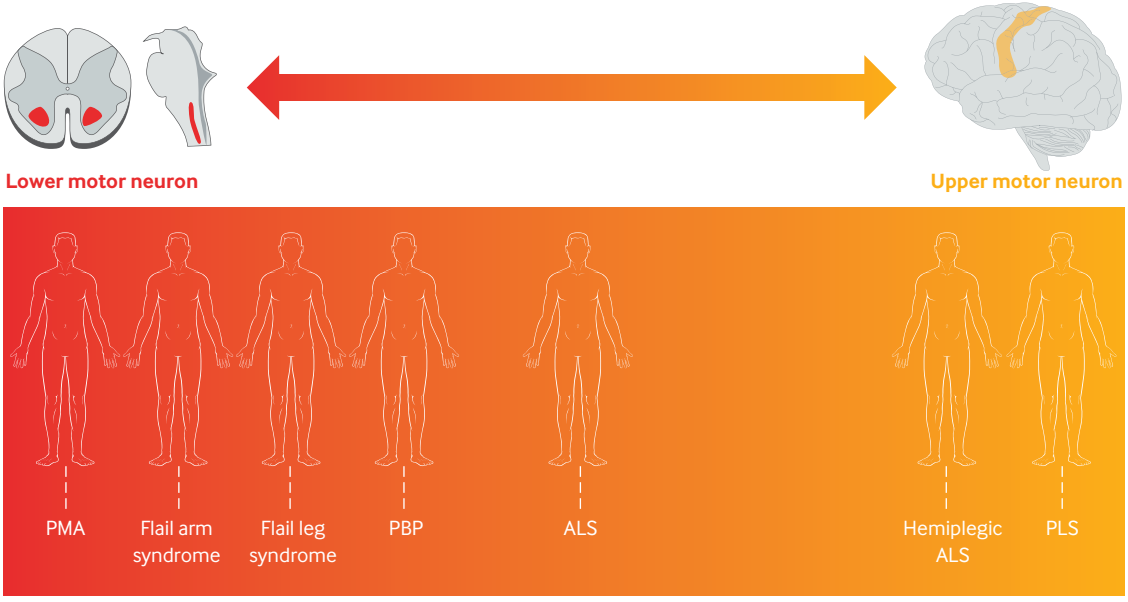


Fig 1 | Amyotrophic lateral sclerosis (ALS) phenotypic spectrum. PBP=progressive bulbar palsy; PLS=primary lateral sclerosis; PMA=progressive muscular atrophy. Created using BioRender.com


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ALS Screen or ALS Cognitive Behavioral Screen are recommended in all patients.[22 23] If the screening test is abnormal, a more extensive neuropsychological evaluation can determine the cognitive and/or behavioral changes.[24] ALS specific behavioral measures such as the Motor Neuron Disease Behavior Scale, the ALS-FTD-Questionnaire, or the Frontal Behavioral Inventory-ALS Version can be used to characterize and assess the severity of behavioral dysfunction.[25-27] The findings of these tests can classify patients as having ALS with cognitive impairment, ALS with behavioral impairment, ALS with combined cognitive and behavioral impairment, ALS with frontotemporal dementia (ALS-FTD) (fig 2),[24] or none of the above (no cognitive or behavioral impairment). Approximately half of all patients with ALS will show impairment on a comprehensive assessment, with approximately 5% classified as ALS with combined cognitive and behavioral impairment, 8% as ALS with behavioral impairment, 17% as ALS with cognitive impairment, and 15-20% as ALS-FTD.[1 28-30]

The most commonly affected cognitive domain in ALS is executive function, with abnormal verbal fluency being a consistent and sensitive marker even after control for bulbar motor dysfunction.[31-33] In patients who develop cognitive symptoms, impaired word fluency is an early finding.[33] Other features of executive dysfunction such as mental inflexibility, inattention and disinhibition, or inability to plan or problem solve can emerge as the disease progresses.[1 20 22] Impairment in multiple cognitive domains is less common in ALS and, when present, tends to involve language or memory and may be confounded by co-pathology such as Alzheimer's disease.[1 34] Isolated amnestic syndrome is not a feature in ALS and should prompt evaluation for an alternative cause. Overall, progression of cognitive

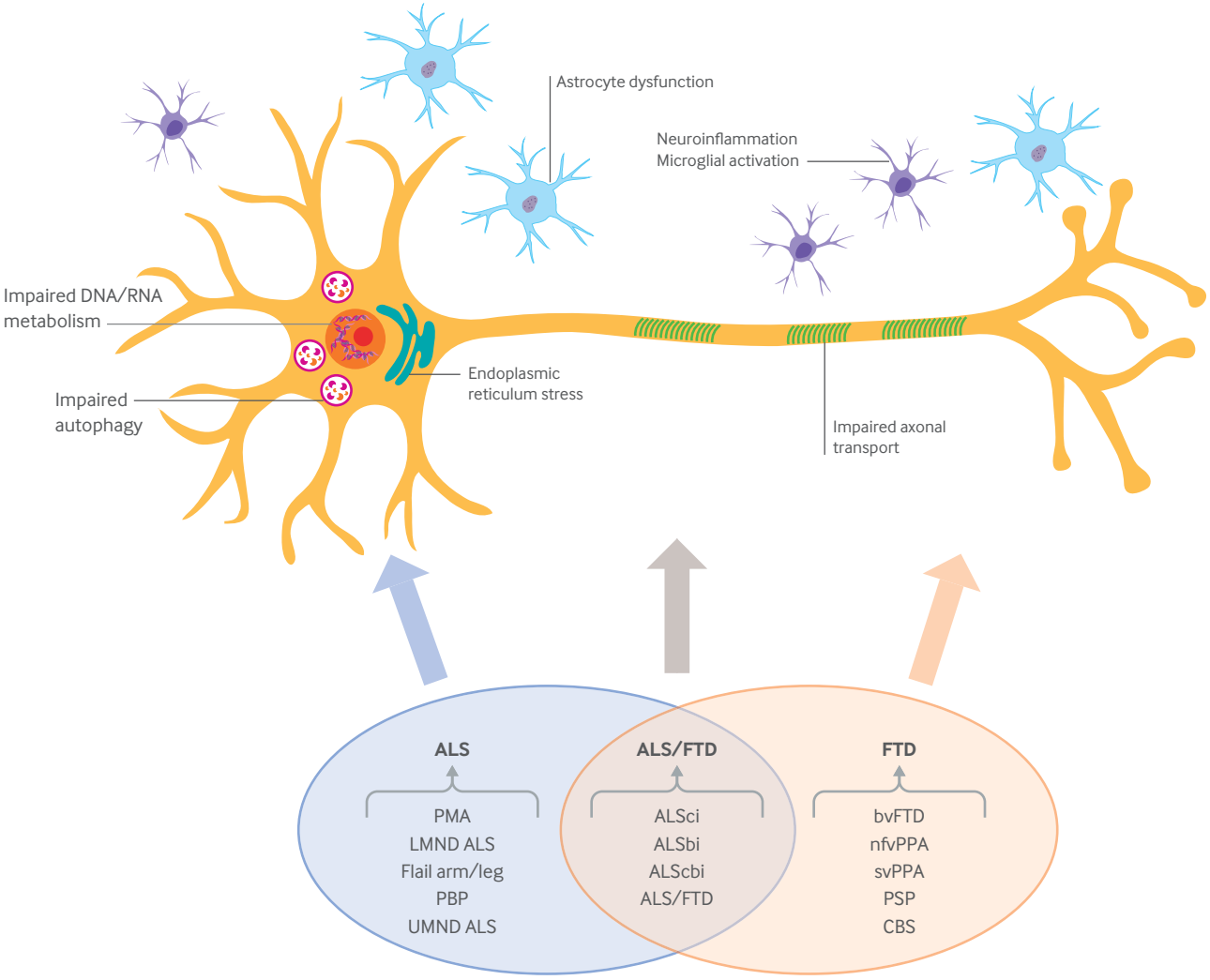


**Fig 2 | Amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD) clinical syndromes and disease mechanisms contributing to neurodegeneration. ALS=amyotrophic lateral sclerosis; ALSbi=ALS with behavioral impairment; ALScbi=ALS with combined cognitive and behavioral impairment; ALSci=ALS with cognitive impairment; bvFTD=behavioral variant FTD; CBS=corticobasal syndrome; FTD=frontotemporal dementia; LMND=lower motor neuron predominant; nfvPPA=non-fluent variant primary progressive aphasia; PBP=progressive bulbar palsy; PLS=primary lateral sclerosis; PMA=progressive muscular atrophy; PSP=progressive supranuclear palsy; svPPA=semantic variant primary progressive aphasia; UMND=upper motor neuron predominant**

dysfunction in ALS is slow and may remain stable over time.[33]

Behavioral abnormalities are a frequent neuropsychiatric feature in ALS, apathy being the most common. Others include disinhibition, perseverative behavior, change in food preferences, loss of empathy, or impaired social cognition including emotional processing.[35-39] Pathological crying and laughing, also known as emotional lability or pseudobulbar affect, is present in approximately one in three patients with ALS and is associated with gray and white matter pathology in the cortico-cerebellar network.[40 41] Pathological crying and laughing does not correlate with neuropsychological measures and should be distinguished from other cognitive and behavioral symptoms.[42]

### Diagnostic criteria and disease progression measures

ALS is a clinical diagnosis requiring findings of progressive motor neuron dysfunction in the absence of an alternative diagnosis. In typical ALS, few tests are needed to support the diagnosis and exclude mimics because other disorders rarely mimic ALS perfectly. The most common tests obtained in the diagnostic process are electrophysiology to establish a lower motor neuronopathy and neuroimaging of the brain and spine to exclude mimics causing structural abnormalities as a cause for UMN dysfunction. Routine laboratory studies are frequently obtained to exclude other causes of a patient's symptoms and are typically normal in ALS. The first widely used criteria in ALS were the El Escorial criteria, which aimed to provide a standardized diagnostic framework to conduct clinical research, with subsequent revisions and updates improving sensitivity allowing for earlier enrollment in clinical trials.[43 44] Although El Escorial and Awaji criteria are useful in clinical research, they are hampered by the heterogeneity of ALS and do not capture the full disease spectrum. For example, cognitive impairment and behavioral impairment are not included in these criteria and pure lower motor neuron variants are excluded.[43] Furthermore, patients with ALS do not necessarily progress through the El Escorial categories of diagnostic certainty and may never attain the criteria for clinically definite ALS.[45] The ALS-frontotemporal spectrum diagnostic (ALS-FTSD) criteria proposed by Strong and colleagues use three diagnostic axes to define MND, cognitive and behavioral dysfunction, and other non-motor features.[24] Although the ALS-FTSD criteria more fully incorporate the ALS-FTD spectrum disorders, they still rely on El Escorial and Awaji criteria to define MND. The recently proposed Gold Coast criteria have attempted to simplify the diagnosis and recognize the potential utility of the development of biomarkers; however, further validation in different populations will be needed before routine use in clinical care or research.[46]

Variability in rate of symptom progression and survival in ALS represents a major obstacle in clinical trials.[47] Disease progression, most often measured by the decline in the revised ALS functional rating scale (ALSFRS-R) over time, differs considerably between patients, and survival ranges from less than one year to more than 10 years.[47 48] Respiratory failure is the most common cause of death. Clinical factors that contribute to heterogeneity in survival include

**Table 1 | Rare causative genes for amyotrophic lateral sclerosis (ALS)**

| Gene (OMIM number) | Protein | Associated clinical diagnosis/feature |
|---|---|---|
| RNA/DNA binding proteins (involved in pre-mRNA processing, metabolism, and transport) | | |
| *TARDBP* (*605078) | TAR DNA binding protein 43 (TDP-43) | ALS with or without FTD; FTD |
| *FUS* (*137070) | Fused in sarcoma protein | ALS with or without FTD; hereditary essential tremor |
| *hnRNPA1* (*164017) and *hnRNPA2B1* (*600124) | Heterogeneous nuclear ribonuclear protein A1 and A2B1 | ALS; inclusion body myopathy with early onset Paget's disease with or without frontotemporal dementia |
| *MATR3* (*164015) | Matrin 3 | ALS with or without cognitive impairment or dementia; distal myopathy |
| *ANG* (*105850) | Angiogenin | Frequent bulbar onset ALS; co-existing parkinsonism with FTD |
| Genes that encode for structural proteins (cytoskeleton proteins) | | |
| *TUBA4A* (*191110) | Tubulin-α 4A (α tubulin) | ALS with or without FTD |
| *ANXA11* (*602572) | Annexin A11 | Later onset ALS (average 67 years); inclusion body myopathy and brain white matter abnormality |
| *PRPH* (*170710) | Peripherin | ALS |
| *DCTN1* (*601143) | Dynactin 1 | Distal motor neuronopathy with vocal paresis |
| *PFN1* (*176610) | Profilin 1 | ALS |
| *KIF5a* (*602821) | KIF5a (kinesin family member 5A) | Also implicated in HSP-10, CMT |
| Loss-of-function mutations in genes encoding proteins important for protein degradation or autophagy pathways | | |
| *UBQLN2* (*300264) | Ubiquilin-2 | ALS with or without FTD; X linked dominant familial ALS; onset younger in males than females |
| *SQSTM1* (*601530) | p62/sequestosome 1 | FTD with or without Paget's disease of the bone; distal myopathy with rimmed vacuoles |
| *OPTN* (*602432) | Optineurin | ALS with or without FTD; allelic with primary open angle glaucoma |
| *VCP* (*601123) | Vasolin containing protein | FTD with or without ALS; inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD); CMT type 2Y |
| *CHMP2B* (*609512) | Charged multivesicular body protein 2B | FTD with or without ALS |
| *VAPB/VAMP* (*605704) | Synatobrevin associated protein B/vesicle associated membrane protein | Lower motor neuronopathy |
| *TBK1* (*604834) | TANK binding kinase 1 | FTD with or without ALS |

CMT=Charcot-Marie-Tooth disease; FTD=frontotemporal dementia; HSP=hereditary spastic paraplegia.

age at symptom onset, sex, site of symptom onset (bulbar versus spinal), time to diagnosis, respiratory measures, pre-symptomatic body mass index, cigarette use, genetics, and the diagnosis of FTD.[49-51] Neurofilament, a biomarker for neurodegeneration, is a predictor of the progression and prognosis of ALS.[52 53] A recently proposed survival prediction model for ALS identified eight prognostic predictors and generated five different survival groups applicable to European patients at the individual level.[54] This model is an important step toward more effective stratification of patients in clinical studies, but validation in non-European groups is needed, and the model will likely evolve as other predictors are identified.

Clinical staging is an important tool for research and care planning because it informs the extent and severity of disease. The two proposed staging systems are the King's staging system and the Milano-Torino staging systems.[55 56] The King's staging system is defined by the number of body regions affected and bulbar and respiratory failure, whereas the Milano-Torino system uses the number of impaired domains as delineated by the ALSFRS-R to define successive stages.[57 58] These systems provide parallel clinical information, using different measures to establish escalating stages, and both have been used to analyze patient population data and are promising endpoints for clinical trials.[58-60] A limitation of both systems is the lack of cognitive and behavioral change captured by staging, although higher disease stage portends more severe cognitive impairment.[29]

## Advances in molecular biology

### Molecular pathology

A new chapter in ALS pathology began in 2006 with the discovery of TDP-43 as the major constituent of ubiquinated aggregates in motor neurons of sporadic ALS and most familial ALS and in cortical neurons in a subgroup of frontotemporal dementia.[5] TDP-43 staining is routinely done in postmortem tissue to characterize the pathology when ALS is suspected.[61] Abnormal accumulation of the protein as either neuronal or glial cytoplasmic inclusions or aggregates is found in 97% of cases of sporadic ALS. Rarely, TDP-43 pathology is not a feature and is seen in ALS caused by superoxidase dismutase 1 (*SOD1*) or fused in sarcoma (*FUS*) gene mutations. Although accumulation of wild type TDP-43 has become the pathological hallmark of ALS, mutations in *TDP-43* are rare and are found in 4-5% of dominantly inherited familial ALS and 1% of sporadic ALS. Additionally, cytoplasmic TDP-43 aggregation can be seen in Alzheimer's disease, atypical parkinsonism, dementia with Lewy bodies, and limbic predominant age related TDP-43 encephalopathy, leading to the recognition of this group of neurodegenerative disorders as TDP-43 proteinopathies.[62 63] TDP-43 was first discovered in 1995, and its function was described as a suppressor of HIV-1 expression. As an RNA/DNA binding protein, it is involved in multiple processes such as RNA processing and maturation, RNA transport, microRNA maturation, and stress granule formation. It normally shuttles between the nucleus and the cytoplasm.[63] The cellular dysfunction leading to TDP-43 aggregation in the cytoplasm and the resultant neurodegeneration is a topic of active research.[64 65] Both loss-of-function and gain-of-function mechanisms have been proposed. An example of the loss-of-function mechanism is the TDP-43 function as a repressor of cryptic exon inclusion.[66] As a result of depletion from the nucleus and loss of the repressor function, cryptic exons (exons that are otherwise excluded from the mRNA) are included in at least two known loci, *STMN2* and *UNC13A*, causing reduced protein expression.[67-70] Of note, cryptic exons are not always shared between species, necessitating the development of new, humanized models. The mild motor neuron degeneration and the inability to replicate the loss of nuclear localization with concomitant cytoplasmic accumulation in animal models have been considered as evidence that these may be late events in the pathogenic cascade.

### Genetics

Whereas the vast majority of ALS is classified as sporadic disease—that is, without known history of another family member with either ALS or frontotemporal dementia—approximately 10% is familial ALS, which can be autosomal dominant, autosomal recessive, or X linked. The list of different causative genes for ALS has grown tremendously (>40 genes), mostly owing to advances in sequencing technologies. Genes causing two thirds of familial ALS and 10% of sporadic ALS are known. Only an overview of the genetics of ALS will be provided here (table 1), as detailed descriptions of all known ALS associated genes are beyond the scope of this review and can be found in other publications.[71]

#### *SOD1*

The earliest understanding of the pathobiology of ALS derived from disease models based on mutations in *SOD1*, the first gene discovered in familial ALS in 1993, which accounts for 20% of familial ALS.[72] Several different mechanisms of neurodegeneration have been proposed, including conformational instability of SOD1 protein, interactions with other proteins, and formation of toxic aggregates, but the exact mechanism remains unclear.[73 74] The consensus is that the many mutations spanning the whole length of *SOD1* confer a toxic gain of function, which has led to development of silencing mutant gene expression as a therapeutic approach.[75 76]

#### *C9ORF72*

The largest genetic contributor to familial ALS was not discovered until 2011 because it is an HRE in an intron of a previously unknown gene named after the region of the chromosome where it is located, *C9ORF72* (chromosome 9, open reading frame 72). Repeats are typically not detectable by standard sequencing methods and require instead repeat

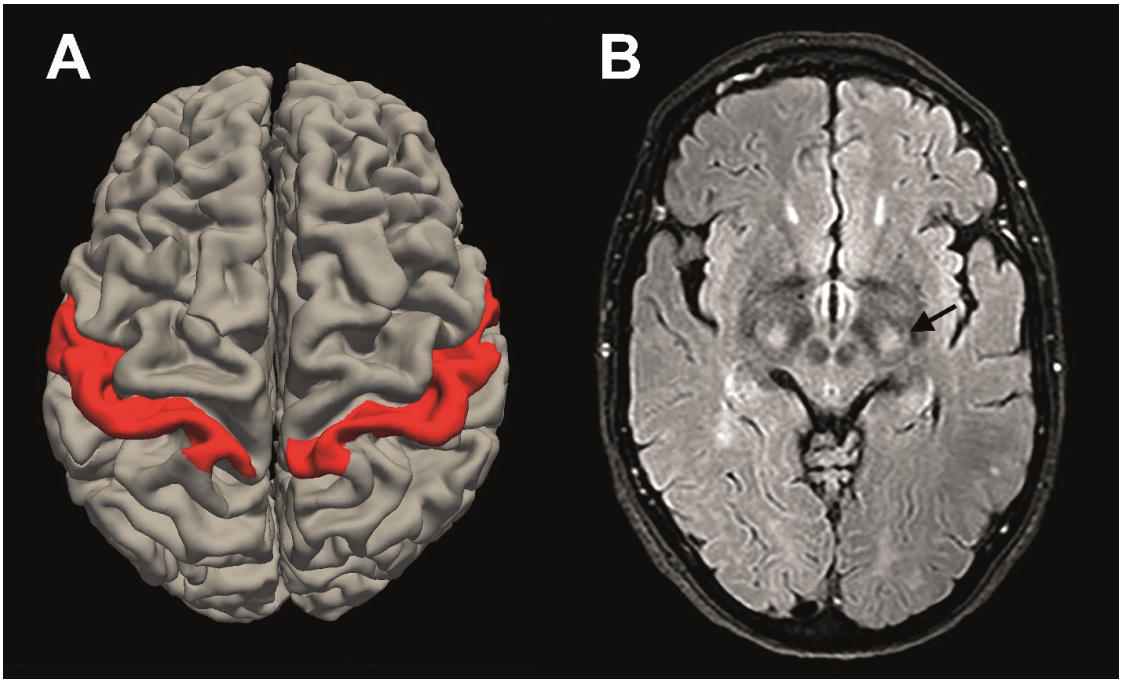


Fig 3 | Neuroimaging changes in amyotrophic lateral sclerosis. A: motor cortex (red) atrophy. B: axial T2-FLAIR (fluid attenuated inversion recovery sequence) magnetic resonance image at level of midbrain, showing hyperintensity in cerebral peduncles corresponding to corticospinal tracts (black arrow)

primed polymerase chain reaction (PCR), a PCR that overcomes the limitations of standard PCR by flanking the repeat region,[77] or a careful analysis of the reason for a drop-off in the sequence coverage of the region in standard next generation sequencing.[4] Intronic nucleotide repeat expansions are known to occur in genes linked to different disorders including myotonic dystrophy, spinocerebellar ataxias (SCA10, SCA 31, SCA 36), and Friedreich's ataxia. In unaffected people, the *C9ORF72* alleles have approximately two to 25 repeats. In contrast, ALS and/or frontotemporal dementia linked to chromosome 9 carry one normal allele and one expanded allele that can have hundreds to thousands of repeats. Somatic instability may further complicate the assessment of the repeat size, as different tissues may have different repeat sizes even within a single patient. The process by which HRE lead to neurodegeneration is not precisely known; however, three major hypotheses have been proposed: the ($G_4C_2$) repeats function similarly to repeat expansions in other disorders (myotonic dystrophy, fragile X tremor/ataxia syndrome) binding and sequestering RNA binding proteins impairing their ability to regulate RNA targets; HRE may cause epigenetic changes resulting in decreased *C9ORF72* mRNA expression; and an atypical mode of polypeptide translation across expanded repeats despite absence of an initiating codon, known as repeat associated non-ATG translation, which is also seen in spinocerebellar ataxia 8 and myotonic dystrophy.[78 79]

### *Epigenetics*

Similar to the growing list of familial ALS linked genes, our knowledge of genetic modifiers in sporadic ALS has increased. Expression levels of genes such as *EphA4*, which encodes a tyrosine kinase receptor that regulates developmental axon outgrowth, inversely correlate with age of disease onset and survival.[80] Variants of *ANG* increase the risk of development of ALS and Parkinson's disease,[81 82] and variants in *NEK-1* (NIMA (never in mitosis gene-A) related kinase-1) were found in nearly 3% of ALS patients.[83] An intermediate length ataxin 2 gene (*ATXN2*) polyglutamine repeats (>23 but <34 polyglutamine repeats) was found in some patients with sporadic ALS, and this finding was later confirmed in additional cohorts.[84-86]

Although interactions between polymorphisms and causative genes of ALS were previously appreciated, the idea that some families with ALS can harbor more than one of these genes was new. Van Blitterwijk et al discovered mutations in more than one ALS linked gene in five out of 97 families.[87] As our knowledge grows, we are likely to find more complex genetic interplay as the basis for disease in individual families.[88] These genes contribute to less than 1% of familial ALS, but their discovery has identified three main cellular functions that, when abnormal, can lead to neuronal degeneration in ALS: RNA/DNA metabolism, protein turnover/autophagy, and cytoskeletal and vesicular regulation (fig 2). This will hopefully improve understanding of disease mechanisms in the search for treatable targets.

## Advances in biomarkers

### Imaging

Neuroimaging is used to look for structural abnormalities in the central nervous system that


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can mimic symptoms and signs of ALS. In most patients with ALS, the brain and spinal cord appear unremarkable or show non-specific abnormalities including corticospinal tract hyperintensity or motor cortex hypointensity in T2 weighted magnetic resonance imaging (MRI) sequences (fig 3).[89 90] Advanced imaging methods are powerful non-invasive research tools that can be used to study and quantify structural, functional, and metabolic abnormalities.[91] For example, voxel based morphometry and surface based morphometry can determine global or regional gray matter atrophy and cortical thinning, and diffusion tensor imaging (DTI) can evaluate the integrity of white matter tracts.[89] Task based and resting state functional MRI can identify differing patterns of blood oxygen level dependent (BOLD) activity to interrogate connectivity neural networks, and magnetic resonance spectroscopy (MRS) allows for quantification of neuronal and glial metabolites such as N-acetylaspartate, a marker of neuronal integrity, creatine, a marker of energy metabolism, choline, a marker of cell membrane, and myo-inositol, a glial marker.[92 93] Other imaging modalities such as positron emission tomography (PET) can show regional changes in brain metabolism by using different receptor ligands.[94] The use of these tools has limitations in research, but technical improvements may prove them useful for group stratification in clinical trials, tracking disease progression, and predicting disease onset in pre-symptomatic carriers of gene mutations. Additionally, they have the potential to provide greater insight into the evolution of pathology.

The most frequent abnormal findings in structural brain imaging studies in ALS are thinning of the motor cortex and atrophy of the precentral gyrus (fig 3) and structural integrity loss in the corticospinal tract and the corpus callosum.[15 95-100] Morphometric changes correlate with the clinical phenotypes and the site of symptom onset, supporting the hypothesis of focal disease onset.[100-102] Another clinical-imaging correlation is the association of cognitive and/or behavioral impairment with extra-motor gray matter volume loss and white matter DTI diffusivity changes.[103-107] The structural abnormalities, however, do not consistently correlate with measures of disease progression such as the ALSFRS-R.[101 103 108-112] More widespread frontal atrophy is associated with faster disease progression and is consistent with the observation that faster disease progression occurs in patients with cognitive and behavioral impairment.[113 114] Longitudinal imaging is invaluable to track disease progression; however, studies show conflicting findings, with a few studies showing progressive gray and white matter changes over time, whereas others show no discernible changes.[97100 102 109 111 115] The causes of these differences include unequal or small sample sizes, clinical heterogeneity, variable follow-up intervals, and different data acquisition and analysis methods. The role of neuroimaging to track clinical progression in ALS remains unresolved, but evolving imaging changes may mirror spread of pathology (for example, TDP-43).[116-119]

In ALS, MRS typically shows decreases in N-acetylaspartate or in N-acetylaspartate/creatine or N-acetylaspartate/choline in the motor cortex and brain stem corresponding to neuronal degeneration.[120-123] MRS indices correlate with clinical UMN disease burden in some studies, but their association with functional and cognitive measures are less consistent.[120-122 124] Additionally, longitudinal MRS studies are often limited by small sample size.[121 125 126] Other metabolites such as γ-aminobutyric acid and glutamate have been examined but need further validation.[122 127]

Early PET studies in ALS using the ligand[18] fluorodeoxyglucose show diffusely reduced uptake in the cortex and deep gray nuclei, mostly in patients who have signs of UMN dysfunction.[128] Other studies show hypometabolism in the frontal regions and hypermetabolism in the temporal regions, cerebellum, and upper brainstem.[129-131] PET ligands binding to the dopamine D2/D3, 5-hydroxytryptamine 1A, and γ-aminobutyric acid A receptors have been examined in ALS, suggesting widespread neuronal dysfunction or degeneration.[132-134] More recently, interest has been growing in examining the role of neuroinflammation in ALS. This has led to the development of PET ligands that bind to the 18-kDa translocator protein expressed by activated glial cells. Studies using this ligand show increased uptake in the primary motor cortex and frontal regions that also show structural and metabolic abnormalities.[135-137] Additional studies are needed to understand the complex interactions between neuronal and glial cells in ALS.

In ALS, task based functional MRI shows increased activation of contralateral, and sometimes ipsilateral, brain regions such as the supplementary motor areas, sensorimotor cortex, temporal regions, deep gray nuclei, and cerebellum.[138-141] These abnormalities are hypothesized to represent adaptive or compensatory responses to the neurodegenerative process. Unlike task based functional MRI, resting state functional MRI shows varying patterns of coherence in the spontaneous BOLD activity. In ALS, the functional connectivity of brain regions can increase, decrease, or be mixed within different brain networks.[15 142 143] The variability in findings can be attributed to methodological differences. Regional decrease in functional connectivity in default mode and sensorimotor networks correlate with greater functional impairment.[143 14]4

## Electrophysiology

### *MUNE, MScanFIT, MUNIX*

Motor unit number estimation (MUNE) is a promising electrophysiological technique to track disease progression in ALS by estimating the number of motor units in a muscle. Distal small muscles such as the intrinsic hand muscles (that is, abductor pollicis brevis) are examined. The concept of MUNE extends from the observation that incremental increases in

the intensity of a stimulus delivered to the motor nerve results in stepwise increases in the amplitude of the compound muscle action potential (CMAP) recorded at the innervated muscle. If the average size of a single motor unit potential contributing to the CMAP can be determined, an estimate of the motor units in that nerve can be calculated by dividing the maximal CMAP amplitude by the average single motor unit potential amplitude.[145] Different methods to determine the average amplitude to calculate MUNE have been developed on the basis of this principle and applied in clinical studies in ALS.[146] Across studies using different methods, MUNE declines with disease progression and correlates with functional rating scales.[147-149]

More recently, the MScanFit MUNE method was developed to estimate the number of motor units from an objective stimulus response curve.[150] MScanFit MUNE may be more accurate, reliable, and easier and quicker to perform and may detect earlier motor neuron loss than other MUNE methods.[151] One limitation is the accessibility of nerves to peripheral stimulation, precluding its use to assess larger proximal muscles. The ease of applying this technique and ability to perform the study using standard electromyography machines make MUNE an attractive biomarker.

Motor unit number index (MUNIX) is an electrophysiological method that estimates the number and size of motor units by recording the maximum CMAP and epochs of surface electromyographic interference pattern at varying force levels.[152] MUNIX can be easily used to assess proximal and distal muscles and has been shown to track disease progression in ALS clinical trials.[153] MUNIX values also decline before development of muscle weakness and may be more sensitive to detect early motor neuron loss.[154 155] However, the inter-rater variability across sites may limit the use of this method in clinical trials.[154]

### *Transcranial magnetic stimulation*

Transcranial magnetic stimulation (TMS) is a non-invasive electrophysiological technique that can objectively assess the integrity of the corticospinal motor neurons. Several different parameters can be measured, including motor threshold, motor evoked amplitude, central motor conduction time, cortical silent period, and intracortical facilitation or inhibition.[156] Compared with controls, the motor threshold is decreased and the motor evoked amplitude is increased in early ALS.[156 157] Other TMS findings include reduced duration of the cortical silent period with increasing stimulation intensity, reduced short intracortical inhibition, and increased intracortical facilitation.[157 158] Collectively, these abnormalities reflect altered cortical excitability. The resting threshold and central motor conduction time have been shown to correlate with clinical findings of UMN dysfunction and are suggested to be useful for tracking disease progression.[126 159] Further studies are needed to establish TMS as a robust biomarker of disease progression.

### *Electrical impedance myography*

Electrical impedance electromyography (EIM) is mostly a research tool to assess the health of the muscle. It is based on recording the voltage that results from applying a weak, high frequency electrical current across sampled muscle without inducing myofiber or neuronal action potentials. The volume conduction properties of the muscle depend on how strongly muscle resists or conducts alternating electrical current (conductivity) and on

**Table 2 | Clinical trials leading to US Food and Drug Administration approved therapies for amyotrophic lateral sclerosis (ALS)**

| Drug, study | Design | No of participants | Primary outcome(s) | Result |
|---|---|---|---|---|
| Riluzole[180] | Prospective, double blind, randomized, placebo controlled | 155 | 1: survival; 2: rate of change in functional status (limb function, bulbar function, muscle testing score) | 1: 45/78 (58%) in placebo group remained alive *v* 57/77 (74%) in riluzole group at 12 months (P=0.014); 2: Rate of deterioration for limb function, bulbar function, and muscle strength was slower in riluzole group, but only statistically significant for muscle testing score (P=0.028) |
| Riluzole[181] | Double blind, randomized, placebo controlled | 959 | Survival without tracheostomy on 100 mg dose | 122 (50.4%) in placebo group and 134 (56.8%) in riluzole group survived; adjusted risk 0.65 (P=0.002) |
| Edaravone[182] | Randomized, double blind, placebo controlled | 137 | Change in ALSFRS-R score from baseline to 24 weeks | Edaravone −5.01 *v* placebo −7.50; least squares mean difference 2.49 (95% CI 0.99 to 3.98; P=0.001) |
| Edaravone[183] | Post hoc analysis of 24 week randomized, placebo controlled study followed by 24 weeks of open label extension study | 88 | Change in ALSFRS-R score at week 48 in patients with FVC ≥80% *v* <80% (FVC assessed at week 24) | FVC >80% subgroup: −7.63 *v* −9.69; difference 2.05 (95% CI 0.16 to 3.94; P=0.034). FVC <80% subgroup: −10.26 *v* −15.20; difference 4.94 (95% CI 1.64 to 8.25; P=0.004). |
| Sodium phenylbutyrate–taurursodiol (PB-TUDCA)[184] | Randomized, double blind; 2:1 (drug:placebo) | 137 | Mean rate of decline in ALSFRS-R at 24 weeks | PB-TUDCA *v* placebo: −1.24/month *v* −1.66/month; difference 0.42 points/month (95% CI 0.03 to 0.81; P=0.03) |
| Tofersen*[185] | Randomized, double blind; 2:1 (drug:placebo) followed by 24 weeks of open label extension study | 108 | Change in ALSFRS-R score at week 28, among participants predicted to have faster progressing disease | Tofersen *v* placebo: −6.98 *v* −8.14; difference 1.2 points (95% CI −3.2 to 5.5; P=0.97). In open label extension (52 weeks), early start *v* delayed start: −6.0 *v* −9.5; difference 3.5 (95% CI 0.4 to 6.7) points |

ALSFRS-R=revised ALS functional rating scale; CI=confidence interval; FVC=forced vital capacity.
*Did not meet primary endpoint.

its ability to store electrical charge within (relative permittivity). These properties have been shown to differ in health and disease in murine models and patients.[160 161] EIM was first used to evaluate the muscle in Duchenne muscular dystrophy, and has more recently been used in ALS.[161] EIM parameters correlate moderately with standard ALS disease progression measures and MUNE. Surface EIM can be done at home, requires minimal training, is painless and repeatable, and has been assessed as an exploratory endpoint in different clinical trials. A motivated patient with ALS can collect surface EIM data more frequently than is done in standard clinical trials, thereby reducing the number of patients needed for a study.[162] An in-depth discussion of the advantages and limitations of both surface and needle EIM can be found in a recent review and subsequent letters to the editor.[163-165] Overall, EIM is a tool in development that may aid ALS patients and researchers to track disease progression.

### Fluid based biomarkers

Significant efforts are being made to evaluate and validate ALS biomarkers of various types (diagnostic, prognostic, predictive, and pharmacodynamic).[166] Neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain have been examined in cerebrospinal fluid and serum in patients with ALS as a marker of neuronal injury.[167-169] Although concentrations are lower in serum than in cerebrospinal fluid, serum is more accessible and can be measured reliably using technologically advanced methods such as single molecule array technology (simoa).[170] In simoa, single molecules are trapped individually in wells followed by a digital readout of beads that are bound to their targets, leading to increased sensitivity for detecting protein at subfemptomolar concentration. Higher neurofilament concentrations tend to correspond to faster disease progression, a feature that can be explored in the design of future clinical trials.[171] A study of pheno-converters (pre-symptomatic carriers of causative genes for ALS who develop symptoms of ALS or frontotemporal dementia) showed increased NfL concentrations occurring at least a year before clinical disease.[169] Once disease begins, serum NfL concentrations are stable longitudinally allowing for its use as a pharmacodynamic marker. More recently, incorporating two related plasma micro-RNAs (mir-181a-5p and mir181b-5p) to NfL concentrations improves the survival prognostication (higher concentrations correlated with shorter survival) especially in the patient group with intermediate (59-109 pg/ml) NfL concentrations.[172] Similar approaches of combining protein(s) and/or RNA(s) biomarkers will be useful owing to enhanced prognostic power.

As an easily accessible biofluid, the urine is an attractive option for screening for biomarkers. The p75 neurotrophin receptor is found on the surface of apoptotic motor neurons and Schwann cells. During normal processing, the ecto domain of the p75 molecule ($p75^{ECD}$) is cleaved and becomes detectable in urine. It is elevated in ALS and increases further with disease progression, thereby making it a putative biomarker.[173] The $p75^{ECD}$, unlike neurofilaments, increases at the time of pheno-conversion and not before, thereby serving as a potential marker of pheno-conversion. As a marker of neuroinflammation, chitinases and related proteins (CHIT-1, CHI3L1, CHI3L2) increase in the cerebrospinal fluid as ALS progresses.[174 175] Although chitinases may be a proxy for neuroinflammation, their use as a biomarker in therapies may be hampered by accessibility (poor serum and cerebrospinal fluid correlation) and polymorphisms that may decrease the protein concentration.[176]

## Advances in ALS therapy

### Multidisciplinary care

The cornerstone of ALS care is an integrative approach because of the clinical and psychosocial complexities.[177 178] A common care model in the US consists of an ALS specialist, nurse, pulmonologist, speech and language pathologist, nutritionist, physical therapist, occupational therapist, and social worker in one clinic visit (as "one stop shop"). Other clinicians with critical roles include a psychiatrist, neuropsychologist, genetics counselor, and gastroenterologist. The ALS team collaborates and seamlessly coordinates care with the primary care clinician and other community or home based health service providers. Additional support is achieved by referral to ALS/MND organizations. This patient centric model of care enhances engagement of patients and care givers in treatment and confers benefits such as improved quality and efficiency of care, access to health and governmental agency services, quality of life, and survival.

### Disease modifying therapies

In the past 20 years, most trials evaluating ALS therapeutics aiming to slow or arrest the neurodegenerative process have failed to show efficacy. These therapies have primarily targeted excitotoxicity, oxidative stress, mitochondrial dysfunction, protein homeostasis, nucleocytoplasmic transport, neuroinflammation, cell death, cytoskeletal integrity, axonal transport, DNA repair, RNA metabolism, and stress granule regulation.[179] As new trials are planned, a collaborative effort has been made to identify contributors to the failure of studies such as clinical and biological heterogeneity. Critical future steps for the global ALS community to accelerate successful development of ALS therapy include ensuring equity of access, optimizing study design and analysis, endpoint harmonization, and data sharing.

Three disease modifying drugs are approved by the US Food and Drug Administration (FDA) with a primary indication for the treatment of ALS (table 2). Riluzole, an anti-glutaminergic drug, increases survival and slows the decline in muscle testing

score.[180 181] The most common side effects are asthenia, gastrointestinal symptoms, and an increase in liver enzymes. Edaravone, a free radical scavenger that acts to decrease oxidative stress, modestly slows ALS disease progression.[182 186] Edaravone is not approved for ALS treatment in Europe, and its role in ALS therapy continues to be a contested topic.[187 188] The combination of sodium phenylbutyrate and taurursodiol targeting mitochondrial dysfunction, endoplasmic reticulum stress, and cell death, was approved by the FDA in 2022.[184]

### Pulmonary intervention

Pulmonary system complications are common in ALS, and respiratory failure is the most frequent cause of death[189] Pulmonary studies relevant in ALS care include spirometry, nocturnal pulse oximetry, arterial blood gas, polysomnography, maximal inspiratory pressure/maximal expiratory pressure, transdiaphragmatic pressure, and sniff nasal pressure.[178 189 190] Serial evaluations are essential to identify respiratory muscle weakness and allow for early interventions using non-invasive ventilation, which has been shown to prolong survival with improved quality of life.[191] Mechanical insufflation-exsufflation is routinely used by ALS patients to augment weak cough to clear airway secretions; however, no systematic study has evaluated the benefits of this intervention. Respiratory muscle training to improve cough and swallowing is an area of active research.[192]

### Diet and nutritional intervention

Weight loss (specifically fat loss) has been shown to correlate with decline in ALSFRS-R scores, and most patients are advised to adapt their diet to maintain a weight close to their premorbid state.[193] Weight loss is multifactorial and associated with decreased food intake due to dysphagia, impaired limb dexterity in handling utensils, hypermetabolism (in about 50% of patients),[194] loss of appetite, and fatigue. Extremes in body mass index (<18, >40) were associated with shorter survival, and best survival was observed for body mass index maintained in the 30-35 range.[195] The consensus is that the diet should include fiber, carotenes, fruits, and antioxidants.[196] However, little consensus exists on the high calorie nutritional source—that is, carbohydrates versus polyunsaturated fats. Clinical guidelines recommend discussion of gastrostomy tube insertion for patients who have symptomatic dysphagia, prolonged eating time, negative caloric balance, unintentional weight loss of greater than 5-10%, and, in some cases, declining respiratory status (forced vital capacity approaching 50%).[189] The benefits of a gastrostomy tube vary depending on proper patient selection, timing of the procedure, careful management of the insertion process, and post-procedure tube management.

ALS patients often ask about over-the-counter supplements and vitamins alone or in combinations. The ALS Untangled (www.alsuntangled.com) initiative has reviewed the evidence for many vitamins and supplements and is an excellent guide for patients, care givers, and clinicians. Unfortunately, most clinical trials have not shown slower ALS progression.[197 198] A recent phase 3 trial of ultrahigh dose methylcobalamin (50 mg) compared with placebo showed a modest slowing in clinical deterioration in treated patients, and evidence suggests that vitamin E may be protective against development of ALS.[199 200]

## Emerging treatments

The modest effects of the current FDA approved therapeutics for such a devastating disease have spurred a growing pipeline of investigational agents. The development of the ALS platform trial allows for simultaneous testing of multiple agents, using a shared master protocol and central infrastructure.[201] Investigation of at least 50 small molecules with various mechanisms is under way. The successes of therapeutics targeting pathogenic gene expression such as antisense oligonucleotides have led to growing interest in this technology in genetic forms of ALS. This is realized in the accelerated approval of tofersen by the FDA for treatment of SOD1-ALS in April 2023. Several phase 1-2 trials are under way examining the benefits of different antisense oligonucleotides targeting *C9ORF72* and *FUS*. Vectors for gene therapy using adeno-associated virus to reduce SOD1 concentrations are also being explored in a phase 1 trial. Monoclonal antibodies targeting misfolded proteins are being evaluated in phase 2 clinical trials.[202]

## Guidelines

The American Academy of Neurology (AAN) practice parameters and European Federation of Neurological Societies (EFNS) guidelines review clinical management of ALS.[177 178 189] The AAN parameters provide a comprehensive, systematic, evidence based review of class I-III studies. However, owing to insufficient evidence for the management of certain symptoms (for example, cramps, spasticity, cognitive/behavioral impairment, pain, and dyspnea), no formal recommendations were made in these domains. The EFNS guidelines include book chapters and review papers, and final recommendations were reached by consensus. Both the AAN and EFNS recommend access to a multidisciplinary center and treatment with riluzole. The EFNS guidelines also cover effective communication of the diagnosis and guidelines for genetic testing. Both the AAN and EFNS recommend percutaneous endoscopic gastrostomy placement for symptom progression and weight stabilization before the vital capacity falls below 50% predicted to minimize procedural related risk. Both guidelines recognize non-invasive ventilation to alleviate symptoms of respiratory insufficiency and to prolong survival. The use of invasive mechanical ventilation is discussed, recognizing that this decision varies according to many factors including economic and


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cultural differences and that, although this prolongs survival, it may not improve quality of life. The AAN practice parameter recognizes the lack of adequate data on drug treatment for cognitive or behavioral impairment in ALS, so no formal recommendations were made.

## Conclusions

In the past 20 years, considerable progress has been made in basic research on ALS. However, this accumulation of knowledge has been slow to translate into effective therapies, a major source of frustration to patients and care givers. With the inclusion of biomarkers, careful and innovative clinical trial design, and targeting of early disease in pre-symptomatic gene mutation carriers, the field is closer to converting basic science discoveries into disease modifying therapies. For the larger group of patients with sporadic ALS, the hope is that by uncovering gene variants that confer risk, and finding methods to define the predominant mechanism (for example, inflammation versus retro-transposon activation versus oxidative stress pathway) as the driver of disease, a personalized approach similar to that for cancer therapeutics can turn ALS into a chronic disease with limited disability and a dignified life.[203]

### GLOSSARY OF ABBREVIATIONS

- AAN—American Academy of Neurology
- ALS—amyotrophic lateral sclerosis
- ALSFRS-R—revised ALS functional rating scale
- ALS-FTD—ALS with frontotemporal dementia
- BOLD—blood oxygen level dependent
- CMAP—compound muscle action potential
- DTI—diffusion tensor imaging
- EFNS—European Federation of Neurological Societies
- EIM—electrical impedance electromyography
- FDA—Food and Drug Administration
- FUS—fused in sarcoma
- HRE—hexanucleotide repeat expansion
- LMN—lower motor neuron
- MND—motor neuron disease
- MRI—magnetic resonance imaging
- MRS—magnetic resonance spectroscopy
- MUNE—motor unit number estimation
- MUNIX—motor unit number index
- NfL—neurofilament light chain
- PCR—polymerase chain reaction
- PET—positron emission tomography
- PLS—primary lateral sclerosis
- PMA—progressive muscular atrophy
- SOD1—superoxidase dismutase 1
- TDP-43—transactive response DNA binding protein 43
- TMS—transcranial magnetic stimulation
- UMN—upper motor neuron

### QUESTIONS FOR FUTURE RESEARCH

- How can diagnostic criteria for amyotrophic lateral sclerosis (ALS) be improved to fully capture motor and cognitive dysfunction?
- What are the genetic contributors to sporadic ALS?
- Can diminishing inflammation in the brain and spinal cord of patients with ALS result in slowing of disease progression?
- What are the major factors driving disease progression, and can they be modified?
- What determines disease presentation (ALS versus frontotemporal dementia (FTD) versus ALS-FTD)?

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1 Phukan J, Elamin M, Bede P, et al. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J Neurol Neurosurg Psychiatry* 2012;83:102-8. doi:10.1136/jnnp-2011-300188
2 Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology* 2002;59:1077-9. doi:10.1212/WNL.59.7.1077
3 Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011;134:2456-77. doi:10.1093/brain/awr179
4 Renton AE, Majounie E, Waite A, et al, ITALSGEN Consortium. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011;72:257-68. doi:10.1016/j.neuron.2011.09.010
5 Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;314:130-3. doi:10.1126/science.1134108
6 Deng HX, Zhai H, Bigio EH, et al. FUS-immunoreactive inclusions are a common feature in sporadic and non-SOD1 familial amyotrophic lateral sclerosis. *Ann Neurol* 2010;67:739-48. doi:10.1002/ana.22051
7 Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers* 2017;3:17071. doi:10.1038/nrdp.2017.71
8 Burrell JR, Halliday GM, Kril JJ, et al. The frontotemporal dementia-motor neuron disease continuum. *Lancet* 2016;388:919-31. doi:10.1016/S0140-6736(16)00737-6
9 Arthur KC, Calvo A, Price TR, Geiger JT, Chiò A, Traynor BJ. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nat Commun* 2016;7:12408. doi:10.1038/ncomms12408
10 Xu L, Liu T, Liu L, et al. Global variation in prevalence and incidence of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J Neurol* 2020;267:944-53. doi:10.1007/s00415-019-09652-y
11 Ravits JM, La Spada AR. ALS motor phenotype heterogeneity, focality, and spread: deconstructing motor neuron degeneration. *Neurology* 2009;73:805-11. doi:10.1212/WNL.0b013e3181b6bbbd
12 van Es MA, Hardiman O, Chio A, et al. Amyotrophic lateral sclerosis. *Lancet* 2017;390:2084-98. doi:10.1016/S0140-6736(17)31287-4

13 Wijesekera LC, Mathers S, Talman P, et al. Natural history and clinical features of the flail arm and flail leg ALS variants. *Neurology* 2009;72:1087-94. doi:10.1212/01.wnl.0000345041.83406.a2
14 Turner MR, Gerhard A, Al-Chalabi A, et al. Mills' and other isolated upper motor neurone syndromes: in vivo study with 11C-(R)-PK11195 PET. *J Neurol Neurosurg Psychiatry* 2005;76:871-4. doi:10.1136/jnnp.2004.047902
15 Verstraete E, van den Heuvel MP, Veldink JH, et al. Motor network degeneration in amyotrophic lateral sclerosis: a structural and functional connectivity study. *PLoS One* 2010;5:e13664. doi:10.1371/journal.pone.0013664
16 Turner MR, Barohn RJ, Corcia P, et al, Delegates of the 2nd International PLS Conference. Primary lateral sclerosis: consensus diagnostic criteria. *J Neurol Neurosurg Psychiatry* 2020;91:373-7. doi:10.1136/jnnp-2019-322541
17 Kim WK, Liu X, Sandner J, et al. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. *Neurology* 2009;73:1686-92. doi:10.1212/WNL.0b013e3181c1dea3
18 de Carvalho M, Scotto M, Swash M. Clinical patterns in progressive muscular atrophy (PMA): a prospective study. *Amyotroph Lateral Scler* 2007;8:296-9. doi:10.1080/17482960701452902
19 Floeter MK, Mills R. Progression in primary lateral sclerosis: a prospective analysis. *Amyotroph Lateral Scler* 2009;10:339-46. doi:10.3109/17482960903171136
20 Crockford C, Newton J, Lonergan K, et al. ALS-specific cognitive and behavior changes associated with advancing disease stage in ALS. *Neurology* 2018;91:e1370-80. doi:10.1212/WNL.0000000000006317
21 Mioshi E, Caga J, Lillo P, et al. Neuropsychiatric changes precede classic motor symptoms in ALS and do not affect survival. *Neurology* 2014;82:149-55. doi:10.1212/WNL.0000000000000023
22 Abrahams S, Newton J, Niven E, Foley J, Bak TH. Screening for cognition and behaviour changes in ALS. *Amyotroph Lateral Scler Frontotemporal Degener* 2014;15:9-14. doi:10.3109/21678421.2013.805784
23 Woolley SC, York MK, Moore DH, et al. Detecting frontotemporal dysfunction in ALS: utility of the ALS Cognitive Behavioral Screen (ALS-CBS). *Amyotroph Lateral Scler* 2010;11:303-11. doi:10.3109/17482961003727954
24 Strong MJ, Abrahams S, Goldstein LH, et al. Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener* 2017;18:153-74. doi:10.1080/21678421.2016.1267768
25 Mioshi E, Hsieh S, Caga J, et al. A novel tool to detect behavioural symptoms in ALS. *Amyotroph Lateral Scler Frontotemporal Degener* 2014;15:298-304. doi:10.3109/21678421.2014.896927
26 Raaphorst J, Beeldman E, Schmand B, et al. The ALS-FTD-Q: a new screening tool for behavioral disturbances in ALS. *Neurology* 2012;79:1377-83. doi:10.1212/WNL.0b013e31826c1aa1
27 Murphy J, Ahmed F, Lomen-Hoerth C. The UCSF screening exam effectively screens cognitive and behavioral impairment in patients with ALS. *Amyotroph Lateral Scler Frontotemporal Degener* 2015;16:24-30. doi:10.3109/21678421.2014.960873
28 Iazzolino B, Pain D, Peotta L, et al. Validation of the revised classification of cognitive and behavioural impairment in ALS. *J Neurol Neurosurg Psychiatry* 2019;90:734-9. doi:10.1136/jnnp-2018-319696
29 Chiò A, Moglia C, Canosa A, et al. Cognitive impairment across ALS clinical stages in a population-based cohort. *Neurology* 2019;93:e984-94. doi:10.1212/WNL.0000000000008063
30 Montuschi A, Iazzolino B, Calvo A, et al. Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. *J Neurol Neurosurg Psychiatry* 2015;86:168-73. doi:10.1136/jnnp-2013-307223
31 Ludolph AC, Langen KJ, Regard M, et al. Frontal lobe function in amyotrophic lateral sclerosis: a neuropsychologic and positron emission tomography study. *Acta Neurol Scand* 1992;85:81-9. doi:10.1111/j.1600-0404.1992.tb04003.x
32 Massman PJ, Sims J, Cooke N, Haverkamp LJ, Appel V, Appel SH. Prevalence and correlates of neuropsychological deficits in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 1996;61:450-5. doi:10.1136/jnnp.61.5.450
33 Abrahams S, Leigh PN, Goldstein LH. Cognitive change in ALS: a prospective study. *Neurology* 2005;64:1222-6. doi:10.1212/01.WNL.0000156519.41681.27
34 Beeldman E, Raaphorst J, Klein Twennaar M, de Visser M, Schmand BA, de Haan RJ. The cognitive profile of ALS: a systematic review and meta-analysis update. *J Neurol Neurosurg Psychiatry* 2016;87:611-9. doi:10.1136/jnnp-2015-310734
35 Grossman AB, Woolley-Levine S, Bradley WG, Miller RG. Detecting neurobehavioral changes in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2007;8:56-61. doi:10.1080/17482960601044106
36 Gibbons ZC, Richardson A, Neary D, Snowden JS. Behaviour in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2008;9:67-74. doi:10.1080/17482960701642437
37 Witgert M, Salamone AR, Strutt AM, et al. Frontal-lobe mediated behavioral dysfunction in amyotrophic lateral sclerosis. *Eur J Neurol* 2010;17:103-10. doi:10.1111/j.1468-1331.2009.02801.x
38 Cuddy M, Papps BJ, Thambisetty M, Leigh PN, Goldstein LH. Processing and memory for emotional and neutral material in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2012;13:592-8. doi:10.3109/17482968.2012.708936
39 Burke T, Pinto-Grau M, Lonergan K, et al. Measurement of Social Cognition in Amyotrophic Lateral Sclerosis: A Population Based Study. *PLoS One* 2016;11:e0160850. doi:10.1371/journal.pone.0160850
40 Floeter MK, Katipally R, Kim MP, et al. Impaired corticopontocerebellar tracts underlie pseudobulbar affect in motor neuron disorders. *Neurology* 2014;83:620-7. doi:10.1212/WNL.0000000000000693
41 Christidi F, Karavasilis E, Ferentinos P, et al. Investigating the neuroanatomical substrate of pathological laughing and crying in amyotrophic lateral sclerosis with multimodal neuroimaging techniques. *Amyotroph Lateral Scler Frontotemporal Degener* 2018;19:12-20. doi:10.1080/21678421.2017.1386689
42 Palmieri A, Abrahams S, Sorarù G, et al. Emotional Lability in MND: Relationship to cognition and psychopathology and impact on caregivers. *J Neurol Sci* 2009;278:16-20. doi:10.1016/j.jns.2008.10.025
43 Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1:293-9. doi:10.1080/146608200300079536
44 Carvalho MD, Swash M. Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. *Amyotroph Lateral Scler* 2009;10:53-7. doi:10.1080/17482960802521126
45 Traynor BJ, Codd MB, Corr B, Forde C, Frost E, Hardiman OM. Clinical features of amyotrophic lateral sclerosis according to the El Escorial and Airlie House diagnostic criteria: A population-based study. *Arch Neurol* 2000;57:1171-6. doi:10.1001/archneur.57.8.1171
46 Shefner JM, Al-Chalabi A, Baker MR, et al. A proposal for new diagnostic criteria for ALS. *Clin Neurophysiol* 2020;131:1975-8. doi:10.1016/j.clinph.2020.04.005
47 Haverkamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. *Brain* 1995;118:707-19. doi:10.1093/brain/118.3.707
48 Swinnen B, Robberecht W. The phenotypic variability of amyotrophic lateral sclerosis. *Nat Rev Neurol* 2014;10:661-70. doi:10.1038/nrneurol.2014.184
49 Chiò A, Logroscino G, Hardiman O, et al, Eurals Consortium. Prognostic factors in ALS: A critical review. *Amyotroph Lateral Scler* 2009;10:310-23. doi:10.3109/17482960802566824
50 Gallo V, Wark PA, Jenab M, et al. Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. *Neurology* 2013;80:829-38. doi:10.1212/WNL.0b013e3182840689
51 Calvo A, Canosa A, Bertuzzo D, et al. Influence of cigarette smoking on ALS outcome: a population-based study. *J Neurol Neurosurg Psychiatry* 2016;87:1229-33. doi:10.1136/jnnp-2016-313793
52 Lu CH, Macdonald-Wallis C, Gray E, et al. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. *Neurology* 2015;84:2247-57. doi:10.1212/WNL.0000000000001642
53 Benatar M, Zhang L, Wang L, et al, CReATe Consortium. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. *Neurology* 2020;95:e59-69. doi:10.1212/WNL.0000000000009559
54 Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. *Lancet Neurol* 2018;17:423-33. doi:10.1016/S1474-4422(18)30089-9
55 Roche JC, Rojas-Garcia R, Scott KM, et al. A proposed staging system for amyotrophic lateral sclerosis. *Brain* 2012;135:847-52. doi:10.1093/brain/awr351
56 Chiò A, Hammond ER, Mora G, Bonito V, Filippini G. Development and evaluation of a clinical staging system for amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2015;86:38-44. doi:10.1136/jnnp-2013-306589
57 Balendra R, Al Khleifat A, Fang T, Al-Chalabi A. A standard operating procedure for King's ALS clinical staging. *Amyotroph Lateral Scler Frontotemporal Degener* 2019;20:159-64. doi:10.1080/21678421.2018.1556696

58 Fang T, Al Khleifat A, Stahl DR, et al, Uk-Mnd LicalS. Comparison of the King's and MiToS staging systems for ALS. *Amyotroph Lateral Scler Frontotemporal Degener* 2017;18:227-32. doi:10.1080/21678421.2016.1265565
59 Balendra R, Jones A, Jivraj N, et al, UK-MND LiCALS Study Group, Mito Target ALS Study Group. Use of clinical staging in amyotrophic lateral sclerosis for phase 3 clinical trials. *J Neurol Neurosurg Psychiatry* 2015;86:45-9. doi:10.1136/jnnp-2013-306865
60 Ferraro D, Consonni D, Fini N, Fasano A, Del Giovane C, Mandrioli J, Emilia Romagna Registry for ALS (ERRALS) Group. Amyotrophic lateral sclerosis: a comparison of two staging systems in a population-based study. *Eur J Neurol* 2016;23:1426-32. doi:10.1111/ene.13053
61 Brettschneider J, Arai K, Del Tredici K, et al. TDP-43 pathology and neuronal loss in amyotrophic lateral sclerosis spinal cord. *Acta Neuropathol* 2014;128:423-37. doi:10.1007/s00401-014-1299-6
62 Lee EB, Lee VM, Trojanowski JQ. Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. *Nat Rev Neurosci* 2011;13:38-50. doi:10.1038/nrn3121
63 de Boer EMJ, Orie VK, Williams T, et al. TDP-43 proteinopathies: a new wave of neurodegenerative diseases. *J Neurol Neurosurg Psychiatry* 2020;92:86-95. doi:10.1136/jnnp-2020-322983
64 Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 2013;79:416-38. doi:10.1016/j.neuron.2013.07.033
65 Birsa N, Bentham MP, Fratta P. Cytoplasmic functions of TDP-43 and FUS and their role in ALS. *Semin Cell Dev Biol* 2020;99:193-201. doi:10.1016/j.semcdb.2019.05.023
66 Ling JP, Pletnikova O, Troncoso JC, Wong PC. TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. *Science* 2015;349:650-5. doi:10.1126/science.aab0983
67 Klim JR, Williams LA, Limone F, et al. ALS-implicated protein TDP-43 sustains levels of STMN2, a mediator of motor neuron growth and repair. *Nat Neurosci* 2019;22:167-79. doi:10.1038/s41593-018-0300-4
68 Melamed Z, López-Erauskin J, Baughn MW, et al. Premature polyadenylation-mediated loss of stathmin-2 is a hallmark of TDP-43-dependent neurodegeneration. *Nat Neurosci* 2019;22:180-90. doi:10.1038/s41593-018-0293-z
69 Brown A-L, Wilkins OG, Keuss MJ, et al. Common ALS/FTD risk variants in UNC13A exacerbate its cryptic splicing and loss upon TDP-43 mislocalization.*bioRxiv* 2021:2021.04.02.438170.
70 Ma XR, Prudencio M, Koike Y, et al. TDP-43 represses cryptic exon inclusion in the FTD-ALS gene UNC13A. *Nature* 2022;603:124-30. doi:10.1038/s41586-022-04424-7
71 Gregory JM, Fagegaltier D, Phatnani H, et al. Genetics of Amyotrophic Lateral Sclerosis. *Curr Genet Med Rep* 2020;8:121-31. doi:10.1007/s40142-020-00194-8 .
72 Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59-62. doi:10.1038/362059a0
73 Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Prog Neurobiol* 2008;85:94-134. doi:10.1016/j.pneurobio.2008.01.001
74 van Zundert B, Brown RHJr. Silencing strategies for therapy of SOD1-mediated ALS. *Neurosci Lett* 2017;636:32-9. doi:10.1016/j.neulet.2016.07.059
75 Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS. *N Engl J Med* 2020;383:109-19. doi:10.1056/NEJMoa2003715
76 Smith RA, Miller TM, Yamanaka K, et al. Antisense oligonucleotide therapy for neurodegenerative disease. *J Clin Invest* 2006;116:2290-6. doi:10.1172/JCI25424
77 DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011;72:245-56. doi:10.1016/j.neuron.2011.09.011
78 Todd TW, Petrucelli L. Insights into the pathogenic mechanisms of Chromosome 9 open reading frame 72 (C9orf72) repeat expansions. *J Neurochem* 2016;138(Suppl 1):145-62. doi:10.1111/jnc.13623
79 Smeyers J, Banchi EG, Latouche M. C9ORF72: What It Is, What It Does, and Why It Matters. *Front Cell Neurosci* 2021;15:661447. doi:10.3389/fncel.2021.661447
80 Van Hoecke A, Schoonaert L, Lemmens R, et al. EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. *Nat Med* 2012;18:1418-22. doi:10.1038/nm.2901
81 Wu D, Yu W, Kishikawa H, et al. Angiogenin loss-of-function mutations in amyotrophic lateral sclerosis. *Ann Neurol* 2007;62:609-17. doi:10.1002/ana.21221
82 Aluri KC, Salisbury JP, Prehn JHM, Agar JN. Loss of angiogenin function is related to earlier ALS onset and a paradoxical increase in ALS duration. *Sci Rep* 2020;10:3715. doi:10.1038/s41598-020-60431-6
83 Kenna KP, van Doormaal PT, Dekker AM, et al, SLAGEN Consortium. NEK1 variants confer susceptibility to amyotrophic lateral sclerosis. *Nat Genet* 2016;48:1037-42. doi:10.1038/ng.3626
84 Lee T, Li YR, Ingre C, et al. Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients. *Hum Mol Genet* 2011;20:1697-700. doi:10.1093/hmg/ddr045
85 Elden AC, Kim HJ, Hart MP, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* 2010;466:1069-75. doi:10.1038/nature09320
86 Narain P, Gomes J, Bhatia R, Singh I, Vivekanandan P. C9orf72 hexanucleotide repeat expansions and Ataxin 2 intermediate length repeat expansions in Indian patients with amyotrophic lateral sclerosis. *Neurobiol Aging* 2017;56:211.e9-14. doi:10.1016/j.neurobiolaging.2017.04.011
87 van Blitterswijk M, van Es MA, Hennekam EA, et al. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum Mol Genet* 2012;21:3776-84. doi:10.1093/hmg/dds199
88 McCann EP, Henden L, Fifita JA, et al. Evidence for polygenic and oligogenic basis of Australian sporadic amyotrophic lateral sclerosis. *J Med Genet* 2020;jmedgenet-2020-106866.
89 Foerster BR, Welsh RC, Feldman EL. 25 years of neuroimaging in amyotrophic lateral sclerosis. *Nat Rev Neurol* 2013;9:513-24. doi:10.1038/nrneurol.2013.153
90 Kwan JY, Jeong SY, Van Gelderen P, et al. Iron accumulation in deep cortical layers accounts for MRI signal abnormalities in ALS: correlating 7 tesla MRI and pathology. *PLoS One* 2012;7:e35241. doi:10.1371/journal.pone.0035241
91 Menke RA, Agosta F, Grosskreutz J, Filippi M, Turner MR. Neuroimaging Endpoints in Amyotrophic Lateral Sclerosis. *Neurotherapeutics* 2017;14:11-23. doi:10.1007/s13311-016-0484-9
92 Turner MR, Agosta F, Bede P, Govind V, Lulé D, Verstraete E. Neuroimaging in amyotrophic lateral sclerosis. *Biomark Med* 2012;6:319-37. doi:10.2217/bmm.12.26
93 Kalra S. Magnetic Resonance Spectroscopy in ALS. *Front Neurol* 2019;10:482. doi:10.3389/fneur.2019.00482
94 Turner MR, Leigh PN. Positron emission tomography (PET)--its potential to provide surrogate markers in ALS. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1(Suppl 2):S17-22. doi:10.1080/54-14660820050515665
95 Agosta F, Pagani E, Rocca MA, et al. Voxel-based morphometry study of brain volumetry and diffusivity in amyotrophic lateral sclerosis patients with mild disability. *Hum Brain Mapp* 2007;28:1430-8. doi:10.1002/hbm.20364
96 Turner MR, Hammers A, Allsop J, et al. Volumetric cortical loss in sporadic and familial amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2007;8:343-7. doi:10.1080/17482960701538734
97 Kwan JY, Meoded A, Danielian LE, Wu T, Floeter MK. Structural imaging differences and longitudinal changes in primary lateral sclerosis and amyotrophic lateral sclerosis. *Neuroimage Clin* 2012;2:151-60. doi:10.1016/j.nicl.2012.12.003
98 Filippini N, Douaud G, Mackay CE, Knight S, Talbot K, Turner MR. Corpus callosum involvement is a consistent feature of amyotrophic lateral sclerosis. *Neurology* 2010;75:1645-52. doi:10.1212/WNL.0b013e3181fb84d1
99 Iwata NK, Kwan JY, Danielian LE, et al. White matter alterations differ in primary lateral sclerosis and amyotrophic lateral sclerosis. *Brain* 2011;134:2642-55. doi:10.1093/brain/awr178
100 van der Graaff MM, Sage CA, Caan MW, et al. Upper and extra-motoneuron involvement in early motoneuron disease: a diffusion tensor imaging study. *Brain* 2011;134:1211-28. doi:10.1093/brain/awr016
101 Schuster C, Kasper E, Machts J, et al. Focal thinning of the motor cortex mirrors clinical features of amyotrophic lateral sclerosis and their phenotypes: a neuroimaging study. *J Neurol* 2013;260:2856-64. doi:10.1007/s00415-013-7083-z
102 Walhout R, Westeneng HJ, Verstraete E, et al. Cortical thickness in ALS: towards a marker for upper motor neuron involvement. *J Neurol Neurosurg Psychiatry* 2015;86:288-94. doi:10.1136/jnnp-2013-306839
103 Prudlo J, Bißbort C, Glass A, et al. White matter pathology in ALS and lower motor neuron ALS variants: a diffusion tensor imaging study using tract-based spatial statistics. *J Neurol* 2012;259:1848-59. doi:10.1007/s00415-012-6420-y
104 Lillo P, Mioshi E, Burrell JR, Kiernan MC, Hodges JR, Hornberger M. Grey and white matter changes across the amyotrophic lateral sclerosis-frontotemporal dementia continuum. *PLoS One* 2012;7:e43993. doi:10.1371/journal.pone.0043993
105 Trojsi F, Corbo D, Caiazzo G, et al. Motor and extramotor neurodegeneration in amyotrophic lateral sclerosis: a 3T high angular resolution diffusion imaging (HARDI) study. *Amyotroph Lateral Scler Frontotemporal Degener* 2013;14:553-61. doi:10.3109/21678421.2013.785569
106 Masuda M, Senda J, Watanabe H, et al. Involvement of the caudate nucleus head and its networks in sporadic amyotrophic lateral sclerosis-frontotemporal dementia continuum. *Amyotroph Lateral Scler Frontotemporal Degener* 2016;17:571-9. doi:10.1080/21678421.2016.1211151

107 Alruwaili AR, Pannek K, Coulthard A, Henderson R, Kurniawan ND, McCombe P. A combined tract-based spatial statistics and voxel-based morphometry study of the first MRI scan after diagnosis of amyotrophic lateral sclerosis with subgroup analysis. *J Neuroradiol* 2018;45:41-8. doi:10.1016/j.neurad.2017.03.007
108 Grosskreutz J, Kaufmann J, Frädrich J, Dengler R, Heinze HJ, Peschel T. Widespread sensorimotor and frontal cortical atrophy in Amyotrophic Lateral Sclerosis. *BMC Neurol* 2006;6:17. doi:10.1186/1471-2377-6-17
109 Zhang Y, Schuff N, Woolley SC, et al. Progression of white matter degeneration in amyotrophic lateral sclerosis: A diffusion tensor imaging study. *Amyotroph Lateral Scler* 2011;12:421-9. doi:10.3109/17482968.2011.593036
110 Iwata NK, Aoki S, Okabe S, et al. Evaluation of corticospinal tracts in ALS with diffusion tensor MRI and brainstem stimulation. *Neurology* 2008;70:528-32. doi:10.1212/01.wnl.0000299186.72374.19
111 Keil C, Prell T, Peschel T, Hartung V, Dengler R, Grosskreutz J. Longitudinal diffusion tensor imaging in amyotrophic lateral sclerosis. *BMC Neurosci* 2012;13:141. doi:10.1186/1471-2202-13-141
112 Müller HP, Turner MR, Grosskreutz J, et al, Neuroimaging Society in ALS (NiSALS) DTI Study Group. A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2016;87:570-9. doi:10.1136/jnnp-2015-311952
113 Mezzapesa DM, D'Errico E, Tortelli R, et al. Cortical thinning and clinical heterogeneity in amyotrophic lateral sclerosis. *PLoS One* 2013;8:e80748. doi:10.1371/journal.pone.0080748
114 Omer T, Finegan E, Hutchinson S, et al. Neuroimaging patterns along the ALS-FTD spectrum: a multiparametric imaging study. *Amyotroph Lateral Scler Frontotemporal Degener* 2017;18:611-23. doi:10.1080/21678421.2017.1332077
115 Cardenas-Blanco A, Machts J, Acosta-Cabronero J, et al. Structural and diffusion imaging versus clinical assessment to monitor amyotrophic lateral sclerosis. *Neuroimage Clin* 2016;11:408-14. doi:10.1016/j.nicl.2016.03.011
116 Verstraete E, Veldink JH, van den Berg LH, van den Heuvel MP. Structural brain network imaging shows expanding disconnection of the motor system in amyotrophic lateral sclerosis. *Hum Brain Mapp* 2014;35:1351-61. doi:10.1002/hbm.22258
117 Kassubek J, Müller HP, Del Tredici K, et al. Diffusion tensor imaging analysis of sequential spreading of disease in amyotrophic lateral sclerosis confirms patterns of TDP-43 pathology. *Brain* 2014;137:1733-40. doi:10.1093/brain/awu090
118 Rosenbohm A, Müller HP, Hübers A, Ludolph AC, Kassubek J. Corticoefferent pathways in pure lower motor neuron disease: a diffusion tensor imaging study. *J Neurol* 2016;263:2430-7. doi:10.1007/s00415-016-8281-2
119 Brettschneider J, Del Tredici K, Toledo JB, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol* 2013;74:20-38. doi:10.1002/ana.23937
120 Bowen BC, Pattany PM, Bradley WG, et al. MR imaging and localized proton spectroscopy of the precentral gyrus in amyotrophic lateral sclerosis. *AJNR Am J Neuroradiol* 2000;21:647-58.
121 Rule RR, Suhy J, Schuff N, Gelinas DF, Miller RG, Weiner MW. Reduced NAA in motor and non-motor brain regions in amyotrophic lateral sclerosis: a cross-sectional and longitudinal study. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2004;5:141-9. doi:10.1080/14660820410017109
122 Atassi N, Xu M, Triantafyllou C, et al. Ultra high-field (7tesla) magnetic resonance spectroscopy in Amyotrophic Lateral Sclerosis. *PLoS One* 2017;12:e0177680. doi:10.1371/journal.pone.0177680
123 Cheong I, Marjańska M, Deelchand DK, Eberly LE, Walk D, Öz G. Ultra-High Field Proton MR Spectroscopy in Early-Stage Amyotrophic Lateral Sclerosis. *Neurochem Res* 2017;42:1833-44. doi:10.1007/s11064-017-2248-2
124 Usman U, Choi C, Camicioli R, et al. Mesial prefrontal cortex degeneration in amyotrophic lateral sclerosis: a high-field proton MR spectroscopy study. *AJNR Am J Neuroradiol* 2011;32:1677-80. doi:10.3174/ajnr.A2590
125 Suhy J, Miller RG, Rule R, et al. Early detection and longitudinal changes in amyotrophic lateral sclerosis by (1)H MRSI. *Neurology* 2002;58:773-9. doi:10.1212/WNL.58.5.773
126 Mitsumoto H, Ulug AM, Pullman SL, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. *Neurology* 2007;68:1402-10. . doi:10.1212/01.wnl.0000260065.57832.87
127 Foerster BR, Carlos RC, Dwamena BA, et al. Multimodal MRI as a diagnostic biomarker for amyotrophic lateral sclerosis. *Ann Clin Transl Neurol* 2014;1:107-14. doi:10.1002/acn3.30
128 Häkkinen S, Chu SA, Lee SE. Neuroimaging in genetic frontotemporal dementia and amyotrophic lateral sclerosis. *Neurobiol Dis* 2020;145:105063. doi:10.1016/j.nbd.2020.105063
129 Van Laere K, Vanhee A, Verschueren J, et al. Value of 18fluorodeoxyglucose-positron-emission tomography in amyotrophic lateral sclerosis: a prospective study. *JAMA Neurol* 2014;71:553-61. doi:10.1001/jamaneurol.2014.62
130 Pagani M, Chiò A, Valentini MC, et al. Functional pattern of brain FDG-PET in amyotrophic lateral sclerosis. *Neurology* 2014;83:1067-74. doi:10.1212/WNL.0000000000000792
131 Pagani M, Öberg J, De Carli F, et al. Metabolic spatial connectivity in amyotrophic lateral sclerosis as revealed by independent component analysis. *Hum Brain Mapp* 2016;37:942-53. doi:10.1002/hbm.23078
132 Lloyd CM, Richardson MP, Brooks DJ, Al-Chalabi A, Leigh PN. Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. *Brain* 2000;123:2289-96. doi:10.1093/brain/123.11.2289
133 Fu X, Zhu W, Guo Z, et al. $^{18}$F-fallypride PET-CT of dopamine D2/D3 receptors in patients with sporadic amyotrophic lateral sclerosis. *J Neurol Sci* 2017;377:79-84. doi:10.1016/j.jns.2017.03.013
134 Turner MR, Rabiner EA, Hammers A, et al. [11C]-WAY100635 PET demonstrates marked 5-HT1A receptor changes in sporadic ALS. *Brain* 2005;128:896-905. doi:10.1093/brain/awh428
135 Zürcher NR, Loggia ML, Lawson R, et al. Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: assessed with [(11)C]-PBR28. *Neuroimage Clin* 2015;7:409-14. doi:10.1016/j.nicl.2015.01.009
136 Alshikho MJ, Zürcher NR, Loggia ML, et al. Integrated magnetic resonance imaging and [$^{11}$ C]-PBR28 positron emission tomographic imaging in amyotrophic lateral sclerosis. *Ann Neurol* 2018;83:1186-97. doi:10.1002/ana.25251
137 Ratai EM, Alshikho MJ, Zürcher NR, et al. Integrated imaging of [$^{11}$C]-PBR28 PET, MR diffusion and magnetic resonance spectroscopy $^{1}$H-MRS in amyotrophic lateral sclerosis. *Neuroimage Clin* 2018;20:357-64. doi:10.1016/j.nicl.2018.08.007
138 Schoenfeld MA, Tempelmann C, Gaul C, et al. Functional motor compensation in amyotrophic lateral sclerosis. *J Neurol* 2005;252:944-52. doi:10.1007/s00415-005-0787-y
139 Konrad C, Jansen A, Henningsen H, et al. Subcortical reorganization in amyotrophic lateral sclerosis. *Exp Brain Res* 2006;172:361-9. doi:10.1007/s00221-006-0352-7
140 Stanton BR, Williams VC, Leigh PN, et al. Altered cortical activation during a motor task in ALS. Evidence for involvement of central pathways. *J Neurol* 2007;254:1260-7. doi:10.1007/s00415-006-0513-4
141 Abidi M, de Marco G, Couillandre A, et al. Adaptive functional reorganization in amyotrophic lateral sclerosis: coexisting degenerative and compensatory changes. *Eur J Neurol* 2020;27:121-8. doi:10.1111/ene.14042
142 Douaud G, Filippini N, Knight S, Talbot K, Turner MR. Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis. *Brain* 2011;134:3470-9. doi:10.1093/brain/awr279
143 Menke RAL, Proudfoot M, Talbot K, Turner MR. The two-year progression of structural and functional cerebral MRI in amyotrophic lateral sclerosis. *Neuroimage Clin* 2017;17:953-61. doi:10.1016/j.nicl.2017.12.025
144 Agosta F, Valsasina P, Absinta M, et al. Sensorimotor functional connectivity changes in amyotrophic lateral sclerosis. *Cereb Cortex* 2011;21:2291-8. doi:10.1093/cercor/bhr002
145 Gooch CL, Doherty TJ, Chan KM, et al. Motor unit number estimation: a technology and literature review. *Muscle Nerve* 2014;50:884-93. doi:10.1002/mus.24442
146 Verber NS, Shepheard SR, Sassani M, et al. Biomarkers in Motor Neuron Disease: A State of the Art Review. *Front Neurol* 2019;10:291. doi:10.3389/fneur.2019.00291
147 Shefner JM, Cudkowicz ME, Zhang H, Schoenfeld D, Jillapalli D, Northeast ALS Consortium. The use of statistical MUNE in a multicenter clinical trial. *Muscle Nerve* 2004;30:463-9. doi:10.1002/mus.20120
148 de Carvalho M, Swash M. Sensitivity of electrophysiological tests for upper and lower motor neuron dysfunction in ALS: a six-month longitudinal study. *Muscle Nerve* 2010;41:208-11.
149 Shefner JM, Watson ML, Simionescu L, et al. Multipoint incremental motor unit number estimation as an outcome measure in ALS. *Neurology* 2011;77:235-41. doi:10.1212/WNL.0b013e318225aabf
150 Bostock H. Estimating motor unit numbers from a CMAP scan. *Muscle Nerve* 2016;53:889-96. doi:10.1002/mus.24945
151 Jacobsen AB, Bostock H, Fuglsang-Frederiksen A, et al. Reproducibility, and sensitivity to motor unit loss in amyotrophic lateral sclerosis, of a novel MUNE method: MScanFit MUNE. *Clin Neurophysiol* 2017;128:1380-8. doi:10.1016/j.clinph.2017.03.045
152 Nandedkar SD, Barkhaus PE, Stålberg EV. Motor unit number index (MUNIX): principle, method, and findings in healthy subjects and in patients with motor neuron disease. *Muscle Nerve* 2010;42:798-807. doi:10.1002/mus.21824
153 Neuwirth C, Barkhaus PE, Burkhardt C, et al. Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): a 15-month longitudinal

multicentre trial. *J Neurol Neurosurg Psychiatry* 2015;86:1172-9. doi:10.1136/jnnp-2015-310509
154 Neuwirth C, Braun N, Claeys KG, et al. Implementing Motor Unit Number Index (MUNIX) in a large clinical trial: Real world experience from 27 centres. *Clin Neurophysiol* 2018;129:1756-62. doi:10.1016/j.clinph.2018.04.614
155 Escorcio-Bezerra ML, Abrahao A, Nunes KF, et al. Motor unit number index and neurophysiological index as candidate biomarkers of presymptomatic motor neuron loss in amyotrophic lateral sclerosis. *Muscle Nerve* 2018;58:204-12. doi:10.1002/mus.26087
156 Vucic S, Ziemann U, Eisen A, Hallett M, Kiernan MC. Transcranial magnetic stimulation and amyotrophic lateral sclerosis: pathophysiological insights. *J Neurol Neurosurg Psychiatry* 2013;84:1161-70. doi:10.1136/jnnp-2012-304019
157 Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain* 2006;129:2436-46. doi:10.1093/brain/awl172
158 Zanette G, Tamburin S, Manganotti P, Refatti N, Forgione A, Rizzuto N. Changes in motor cortex inhibition over time in patients with amyotrophic lateral sclerosis. *J Neurol* 2002;249:1723-8. doi:10.1007/s00415-002-0926-7
159 Floyd AG, Yu QP, Piboolnurak P, et al. Transcranial magnetic stimulation in ALS: utility of central motor conduction tests. *Neurology* 2009;72:498-504. doi:10.1212/01.wnl.0000341933.97883.a4
160 Nagy JA, DiDonato CJ, Rutkove SB, Sanchez B. Permittivity of ex vivo healthy and diseased murine skeletal muscle from 10 kHz to 1 MHz. *Sci Data* 2019;6:37. doi:10.1038/s41597-019-0045-2
161 Rutkove SB, Sanchez B. Electrical Impedance Methods in Neuromuscular Assessment: An Overview. *Cold Spring Harb Perspect Med* 2019;9:a034405. doi:10.1101/cshperspect.a034405
162 Rutkove SB, Narayanaswami P, Berisha V, et al. Improved ALS clinical trials through frequent at-home self-assessment: a proof of concept study. *Ann Clin Transl Neurol* 2020;7:1148-57. doi:10.1002/acn3.51096
163 Sanchez B. Reply to "Putting the patient first: The validity and value of surface-based electrical impedance myography techniques". *Clin Neurophysiol* 2021;132:1754-5. doi:10.1016/j.clinph.2021.03.019
164 Rutkove SB, Narayanaswami P, David Arnold W, et al. Putting the patient first: The validity and value of surface-based electrical impedance myography techniques. *Clin Neurophysiol* 2021;132:1752-3. doi:10.1016/j.clinph.2021.03.020
165 Sanchez B, Martinsen OG, Freeborn TJ, Furse CM. Electrical impedance myography: A critical review and outlook. *Clin Neurophysiol* 2021;132:338-44. doi:10.1016/j.clinph.2020.11.014
166 Benatar M, Boylan K, Jeromin A, et al. ALS biomarkers for therapy development: State of the field and future directions. *Muscle Nerve* 2016;53:169-82. doi:10.1002/mus.24979
167 Gendron TF, Daughrity LM, Heckman MG, et al, C9ORF72 Neurofilament Study Group. Phosphorylated neurofilament heavy chain: A biomarker of survival for C9ORF72-associated amyotrophic lateral sclerosis. *Ann Neurol* 2017;82:139-46. doi:10.1002/ana.24980
168 Boylan KB, Glass JD, Crook JE, et al. Phosphorylated neurofilament heavy subunit (pNF-H) in peripheral blood and CSF as a potential prognostic biomarker in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2013;84:467-72. doi:10.1136/jnnp-2012-303768
169 Benatar M, Wuu J, Andersen PM, Lombardi V, Malaspina A. Neurofilament light: A candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. *Ann Neurol* 2018;84:130-9. doi:10.1002/ana.25276
170 Rissin DM, Kan CW, Campbell TG, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol* 2010;28:595-9. doi:10.1038/nbt.1641
171 Poesen K, De Schaepdryver M, Stubendorff B, et al. Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease. *Neurology* 2017;88:2302-9. doi:10.1212/WNL.0000000000004029
172 Magen I, Yacovzada NS, Yanowski E, et al. Circulating miR-181 is a prognostic biomarker for amyotrophic lateral sclerosis. *Nat Neurosci* 2021;24:1534-41. doi:10.1038/s41593-021-00936-z
173 Shepheard SR, Chataway T, Schultz DW, Rush RA, Rogers ML. The extracellular domain of neurotrophin receptor p75 as a candidate biomarker for amyotrophic lateral sclerosis. *PLoS One* 2014;9:e87398. doi:10.1371/journal.pone.0087398
174 Vu L, An J, Kovalik T, Gendron T, Petrucelli L, Bowser R. Cross-sectional and longitudinal measures of chitinase proteins in amyotrophic lateral sclerosis and expression of CHI3L1 in activated astrocytes. *J Neurol Neurosurg Psychiatry* 2020;91:350-8. doi:10.1136/jnnp-2019-321916
175 Gray E, Thompson AG, Wuu J, et al. CSF chitinases before and after symptom onset in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol* 2020;7:1296-306. doi:10.1002/acn3.51114
176 Boot RG, Renkema GH, Verhoek M, et al. The human chitotriosidase gene. Nature of inherited enzyme deficiency. *J Biol Chem* 1998;273:25680-5. doi:10.1074/jbc.273.40.25680
177 Miller RG, Jackson CE, Kasarskis EJ, et al, Quality Standards Subcommittee of the American Academy of Neurology. Practice parameter update: the care of the patient with amyotrophic lateral sclerosis: multidisciplinary care, symptom management, and cognitive/behavioral impairment (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2009;73:1227-33. doi:10.1212/WNL.0b013e3181bc01a4
178 Andersen PM, Abrahams S, Borasio GD, et al, EFNS Task Force on Diagnosis and Management of Amyotrophic Lateral Sclerosis. EFNS guidelines on the clinical management of amyotrophic lateral sclerosis (MALS)--revised report of an EFNS task force. *Eur J Neurol* 2012;19:360-75. doi:10.1111/j.1468-1331.2011.03501.x
179 Chiò A, Mazzini L, Mora G. Disease-modifying therapies in amyotrophic lateral sclerosis. *Neuropharmacology* 2020;167:107986. doi:10.1016/j.neuropharm.2020.107986
180 Bensimon G, Lacomblez L, Meininger V, ALS/Riluzole Study Group. A controlled trial of riluzole in amyotrophic lateral sclerosis. *N Engl J Med* 1994;330:585-91. doi:10.1056/NEJM199403033300901
181 Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V, Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. *Lancet* 1996;347:1425-31. doi:10.1016/S0140-6736(96)91680-3
182 Writing Group, Edaravone (MCI-186) ALS 19 Study Group. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol* 2017;16:505-12. doi:10.1016/S1474-4422(17)30115-1
183 Brooks BR, Heiman-Patterson T, Wiedau-Pazos M, Liu S, Zhang J, Apple S. Edaravone efficacy in amyotrophic lateral sclerosis with reduced forced vital capacity: Post-hoc analysis of Study 19 (MCI186-19) [clinical trial NCT01492686][clinical trial NCT01492686]. *PLoS One* 2022;17:e0258614. doi:10.1371/journal.pone.0258614
184 Paganoni S, Macklin EA, Hendrix S, et al. Trial of Sodium Phenylbutyrate-Taurursodiol for Amyotrophic Lateral Sclerosis. *N Engl J Med* 2020;383:919-30. doi:10.1056/NEJMoa1916945
185 Miller TM, Cudkowicz ME, Genge A, et al, VALOR and OLE Working Group. Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS. *N Engl J Med* 2022;387:1099-110. doi:10.1056/NEJMoa2204705
186 Takahashi F, Takei K, Tsuda K, Palumbo J. Post-hoc analysis of MCI186-17, the extension study to MCI186-16, the confirmatory double-blind, parallel-group, placebo-controlled study of edaravone in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2017;18(sup1):32-9. doi:10.1080/21678421.2017.1361442
187 Al-Chalabi A, Andersen PM, Chandran S, et al. July 2017 ENCALS statement on edaravone. *Amyotroph Lateral Scler Frontotemporal Degener* 2017;18:471-4. doi:10.1080/21678421.2017.1369125
188 Glass JD, Fournier CN. Unintended Consequences of Approving Unproven Treatments-Hope, Hype, or Harm?*JAMA Neurol* 2022;79:117-8. doi:10.1001/jamaneurol.2021.4193
189 Miller RG, Jackson CE, Kasarskis EJ, et al, Quality Standards Subcommittee of the American Academy of Neurology. Practice parameter update: the care of the patient with amyotrophic lateral sclerosis: drug, nutritional, and respiratory therapies (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2009;73:1218-26. doi:10.1212/WNL.0b013e3181bc0141
190 Lechtzin N, Cudkowicz ME, de Carvalho M, et al. Respiratory measures in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2018;19:321-30. doi:10.1080/21678421.2018.1452945
191 Bourke SC, Tomlinson M, Williams TL, Bullock RE, Shaw PJ, Gibson GJ. Effects of non-invasive ventilation on survival and quality of life in patients with amyotrophic lateral sclerosis: a randomised controlled trial. *Lancet Neurol* 2006;5:140-7. doi:10.1016/S1474-4422(05)70326-4
192 Plowman EK, Watts SA, Tabor L, et al. Impact of expiratory strength training in amyotrophic lateral sclerosis. *Muscle Nerve* 2016;54:48-53. doi:10.1002/mus.24990
193 Lee I, Kazamel M, McPherson T, et al. Fat mass loss correlates with faster disease progression in amyotrophic lateral sclerosis patients: Exploring the utility of dual-energy x-ray absorptiometry in a prospective study. *PLoS One* 2021;16:e0251087. doi:10.1371/journal.pone.0251087
194 Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. *Lancet Neurol* 2011;10:75-82. doi:10.1016/S1474-4422(10)70224-6
195 Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills AM. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. *Muscle Nerve* 2011;44:20-4. doi:10.1002/mus.22114

196 Nieves JW, Gennings C, Factor-Litvak P, et al, Amyotrophic Lateral Sclerosis Multicenter Cohort Study of Oxidative Stress (ALS COSMOS) Study Group. Association Between Dietary Intake and Function in Amyotrophic Lateral Sclerosis. *JAMA Neurol* 2016;73:1425-32. doi:10.1001/jamaneurol.2016.3401
197 Prell T, Grosskreutz J, Pooled Resource Open-Access ALS Clinical Trials Consortium. Use of vitamins by participants in amyotrophic lateral sclerosis clinical trials. *PLoS One* 2020;15:e0237175. doi:10.1371/journal.pone.0237175
198 Kaufmann P, Thompson JL, Levy G, et al, QALS Study Group. Phase II trial of CoQ10 for ALS finds insufficient evidence to justify phase III. *Ann Neurol* 2009;66:235-44. doi:10.1002/ana.21743
199 Oki R, Izumi Y, Fujita K, et al, Japan Early-Stage Trial of Ultrahigh-Dose Methylcobalamin for ALS (JETALS) Collaborators. Efficacy and Safety of Ultrahigh-Dose Methylcobalamin in Early-Stage Amyotrophic Lateral Sclerosis: A Randomized Clinical Trial. *JAMA Neurol* 2022;79:575-83. doi:10.1001/jamaneurol.2022.0901
200 Wang H, O'Reilly EJ, Weisskopf MG, et al. Vitamin E intake and risk of amyotrophic lateral sclerosis: a pooled analysis of data from 5 prospective cohort studies. *Am J Epidemiol* 2011;173:595-602. doi:10.1093/aje/kwq416
201 Paganoni S, Berry JD, Quintana M, et al, Healey ALS Platform Trial Study Group. Adaptive Platform Trials to Transform Amyotrophic Lateral Sclerosis Therapy Development. *Ann Neurol* 2022;91:165-75. doi:10.1002/ana.26285
202 Johnson SA, Fang T, De Marchi F, et al. Pharmacotherapy for Amyotrophic Lateral Sclerosis: A Review of Approved and Upcoming Agents. *Drugs* 2022;82:1367-88. doi:10.1007/s40265-022-01769-1
203 Tam OH, Rozhkov NV, Shaw R, et al, NYGC ALS Consortium. Postmortem Cortex Samples Identify Distinct Molecular Subtypes of ALS: Retrotransposon Activation, Oxidative Stress, and Activated Glia. *Cell Rep* 2019;29:1164-1177.e5. doi:10.1016/j.celrep.2019.09.066