[Au: We really need to reduce the number of references to ~250; accordingly, I have suggested a few places in the manuscript where the number of references could be reduced. I have reduced this to 256]

Amyotrophic lateral sclerosis

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Competing interests [Au: Please note I've restructured the order of the CIs here so they follow the same order as the author list]

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[Au: Please add the competing interests for Emma Corr and Wim here - if these authors do not have any competing interests, this should be added here. Our competing interest policy can be found here: http://www.nature.com/authors/policies/competing.html. Essentially, competing financial interests include honoraria, consultation fees, research grants, stocks, etc. from for-profit companies. Emma Corr and Wim do not have any competing interests] .

Author contributions

Introduction (O.H.); Epidemiology (G.L.); Mechanisms/pathophysiology, (W.R. and P.J.S.); Genetics, Diagnosis, screening and prevention, (O.H and L.H.B.); Management, (A.C.); Quality of life, (Z.S.); Outlook, (A.A.); Overview of Primer, (E.M.C. and O.H.).

Abstract Amyotrophic lateral sclerosis (ALS), also known as Motor Neuron Disease (MND) **[? It is synonymous.]**, is characterized by the degeneration of both upper and lower motor neurons, leading to muscle weakness and eventual paralysis. Until recently, ALS was classified primarily within the

neuromuscular domain, although new imaging and neuropathological data have indicated the involvement of the non-motor neuraxis in disease pathology. In most patients, the mechanisms underlying development of ALS are poorly understood, although a subset of patients have familial disease and carry mutations in genes that have various roles in neuronal function. Two disease modifying therapies which can slow disease progression, are available for the treatment of ALS, but patient management is largely mediated by the use of symptomatic therapies, such as the use of muscle relaxants for spasticity and speech therapy for dysarthria.

[H1] Introduction

Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative syndrome that is characterized by the degeneration of both upper (that is, neurons that project from the cortex to the brain stem and the spinal cord) and lower (that is, neurons that project from the brainstem or spinal cord to the muscle) motor neurons leading to motor and extra-motor symptoms (Figure 1). The initial presentation of ALS can vary between patients; some present with spinal-onset disease (that is, the onset of muscle weakness of the limbs), but others can present with bulbar-onset disease (characterized by dysarthria – difficulty with speech – and dysphagia – difficulty swallowing. In most patients, the cause of ALS is unknown, although some individuals develop familial forms of the disease, which are associated with mutations in genes that have a wide range of functions, including functions in non-motor cells. In the familial forms of the disease, some of the implicated genes are incompletely penetrant, and with rare exceptions, genotype does not necessarily predict phenotype [1]. Although the primary symptoms of ALS are associated with motor dysfunction (such as muscle weakness, spasticity and dysphagia), up to 50% of patients develop cognitive and/or behavioral impairment during the course of disease and 13% of patients present with concomitant behavioral variant frontotemporal dementia (bv-FTD)[2-4]. The high prevalence of cognitive and/or behavioural symptoms, coupled with the finding of a hexanucleotide repeat expansion in *C9orf72* as the major genetic cause of ALS and FTD [5, 6], have contributed to the re-characterization of ALS as a neurodegenerative, rather than a neuromuscular disorder, and have signposted the direction of research over the coming decade.

The classification of ALS can vary depending on the criteria used. The traditional definitions of ALS subgroups are based on the extent of upper and lower motor neuron involvement, although other classification systems include different parameters, such as the site of onset (that is, bulbar or spinal

onset of disease), the level of certainty of diagnosis according to the revised El Escorial Criteria and heritability (sporadic or familial disease)[7]. To date, none of these classification systems have incorporated the cognitive or behavioural symptoms and within each classification system a range of sub-phenotypes and clinical trajectories can be demonstrated.

This Primer will review the aspects of ALS that contribute to disease heterogeneity, and will look to the future of new therapeutic trials that incorporate recent advances in our understanding of this disease spectrum. For new therapies, the challenge is to define mechanisms of disease amenable to drug targeting, and to define sub-cohorts of patients that are likely to respond to these new therapeutic agents.

[H1] Epidemiology

[H2] Descriptive epidemiology

The majority of population based epidemiological studies for ALS have come from high quality European patient Registers [8]. These European population based Registers have been combined to form the European ALS Epidemiology Consortium (EURALS), which has provided data comparing the incidence of ALS between European countries [9]. In Europe, the incidence ranges from 2-3 cases per 100,000 individuals**.** Defined geographical areas are ideally suited to estimate the incidence and prevalence, and to support more-detailed studies of risk, clinical trajectory, outcome and utilization of services for ALS[8]. As ALS is a rare disease, a population-based approach with multiple sources of ascertainment is the best way to describe the entire phenotypic spectrum [10] as population-based registers provide more complete information about the disease than datasets from specialist clinics, which are often biased in favour of younger patients and those with less severe disease **[?OK]** [10]. Similarly, clinical trial cohorts such as those collected within the US-based pooled resource open-access ALS clinical trials database (**?OK]** ProACT) dataset also select for patients with ALS who have better prognosis; survival within these cohorts is ~12 months longer than that of true population-based cohorts.

[OK] [OK] Contrary to earlier assumptions, the incidence of ALS has been shown to differ based on ancestral origin; studies in populations of European origin **[]** have shown a crude incidence of >3 cases per 100,000 individuals [11, 12], but incidence rates are lower in East Asia (around 0.8 per 100,00) and

South Asia (0.7 per 100,000). In some regions (such as Guam and the Kii peninsula of Japan) the reported incidence was very high, but dropped substantially over the past 30 years for reasons that remain unclear. In areas where different ancestral populations live in close proximity (as in Northern America), the incidence rates of ALS in indigenous populations is particularly low (0.63 cases per 100,000 individuals)[13], whereas reported incidences in regions of relatively homogeneous populations (such as Ireland, Scotland and the Faroe Islands) are high (2.6 cases per 100, 000 individuals) [9, 14].

In addition, variations in the phenotype and natural history of ALS have been reported in different ancestral populations; indeed reported survival of patients with ALS is much shorter in Europe (24 months) than in Central Asia (48 months) [15]. **[OK]** In addition, admixed populations (that is, populations of mixed ancestry **OK]**) might have lower mortality rates of ALS. In a population-based study in Cuba, ALS mortality rate was 0.55 per 100,000 individuals in a mixed population,**[OK]** but was about 0.9 per 100,000 individuals **[Au:OK?OK]** in white or black individuals [16], confirming the importance of ancestral origin in disease risk. **[ok]** In Europe, most men have spinal onset disease, and women have increased propensity for bulbar onset disease [9]. The percentage of individuals with bulbar onset disease is much lower in Asia compared with Europe, but a North to South gradient has been described in Europe, with higher percentage of individuals with spinal onset disease in Southern Europe [9]. Based on available data, the age of diagnosis and first symptoms is higher in Europe compared to Asia and South America. **[OK]** In Europe, the age of onset peaks at 65 [9]. **[]** The main limitation of global ALS epidemiology is that almost 80% of studies have been conducted in Europe and the US, and mainly comprise patient cohorts of Northern European ancestry. International consortia collecting data in areas with mixed populations and in different continents will be required to fully elucidate the range of clinical presentations, and to understand the roles of ancestry, genetics and environmental exposures in ALS causation.

[H2] Causes of ALS

[H3] Genetics. ALS is considered a complex genetic disorder with a Mendelian pattern of inheritance in a proportion of cases, but no discernible family history in the rest. Mathematical models developed using population-based registers have suggested that individuals with ALS are likely to carry a number of 'at risk' variants that interact with environmental factors through a series of at least 6 notional steps leading to disease manifestation. One of these steps is thought to be the genetic risk (from birth), but the interplay of environmental factors that lead to the remaining steps have yet to be defined. In

transgenic mice, the genetic background can alter the phenotypic presentation of ALS **[OK ?]** [17, 18], suggesting that human disease phenotypes could also have a genetic basis, and that genomic and epigenomic "fingerprinting" could permit the clustering of different phenotypic manifestations into discrete underlying causes that are amenable to therapeutic intervention.

Large combined genome-wide association studies (GWAS) of apparently sporadic ALS suggest that the genetic architecture is based primarily on rare variants, in contrast to other diseases, such as schizophrenia, which are associated with large numbers of common variants. GWAS in ALS are also complicated as the rare variants that confer risk might be specific to individuals, families and ancestral populations [19], rendering GWAS less suited for study of ALS genetics than is schizophrenia. Initiatives such as the Project MinE Consortium (www.projectmine.com), which aims to undertake whole genome sequencing of >16,000 patients with ALS and 6,000 control individuals, are likely to provide greater clarity of the genetic architecture of ALS.

Of the known genes of major effect for the development of ALS (Table 1 **[** OK), our current knowledge comes primarily from the study of ancestral European (Europe, USA, Canada and Australia) and East Asian populations; within these populations, **OK**the dichotomization of ALS into 'familial' and 'sporadic' subtypes is an over-simplification. Although at least 30 genes are known to confer a major risk for ALS, evidence suggests a role of oligogenic inheritance (in which a phenotypic trait is determined by more than one gene**? OK]**) and of genetic pleiotropy (in which a single gene **[OK]** has multiple phenotypic manifestations). Within populations of European extraction, up to 20% of people with ALS have a family history of either ALS or FTD (Familial ALS) , and of these 4 genes account for up to 70% of all cases of familial ALS , namely *C9orf72*, *TARDBP* (also known as *TDP43*), *SOD1* and *FUS* **[?OK]** [20]. However, even in the case of these known Mendelian inherited genes, familial forms of ALS are often characterized by lower than 50% penetrance **[**and genetic pleiotropy, with evidence of oligogenic and polygenic inheritance in individuals with apparently sporadic disease [21, 22].

[H3] Environmental and lifestyle factors. OK]. Epidemiological case control studies have sought to determine the environmental causes of ALS. Early epidemiological studies from regions with a high incidence of ALS and dementia such as Guam and the Kii peninsula of Japan suggested a role for neurotoxins **[Would prefer to retain neurotoxins]** contained within cycad seeds, including β-methylamino-L-alanine **OK]** . Although the role of β-methylamino-L-alanine[23] has not been

substantiated, a possible role for related cyanotoxins has been proposed, and exposure to water harbouring cyanobacterial blooms has been suggested to contribute to risk of ALS in susceptible individuals [24].

ALS has been reported at a higher frequency among groups of athletes compared to the general population although whether physical activity is a risk factor for ALS, or a marker of underlying athletic prowess is unclear. Evidence from a UK study suggests that individuals with ALS had higher rates of pre-morbid **[Pre-Morbid is ok – standard use PJS I AGREE]** physical activity, but two other European studies suggested either no effect, or a protective effect [22-24]. Reasons for this discrepancy **[**might relate to study design and true population-based differences. However, because ALS is a rare disease, smaller case control studies are often underpowered and are subject to both bias and error in interpretation. To address these problems in study design, a very large case control study **OK** has been completed as part of the EuroMOTOR project (www.euromotorproject.eu*)*, which has collected >1,500 population-based incident cases and 3,000 matched controls across 3 countries. Analysis is ongoing, although preliminary data suggest that exposure to smoking might increase the risk of developing ALS, but type 2 diabetes mellitus, high levels of circulating lipids and exposure to **[EXPOSURE is more accurate** female contraceptive hormones seem to be protective [25, 26] **[(YES- OH is the senior author) PENDING** .

[H1] Mechanisms/pathophysiology

[H2] Histopathology [?OK]

Although the fundamental pathophysiological mechanisms underlying ALS are not well understood, the neuropathological hallmark of disease is the aggregation and accumulation of ubiquitinated proteinaceous inclusions in motor neurons **[?YES]** . Protein inclusions occur in other neurodegenerative disorders (such as amyloid plaques in Alzheimer Disease and synuclein-containing Lewy Bodies in Parkinson Disease **OK]**. The biological processes leading to formation of these inclusions **OK]** has been the subject of intensive research, but is poorly understood [4].

In most subtypes of ALS the tar DNA-binding protein 43 **OK]** (TDP-43) is the major constituent of these inclusions, although mutations in *TARDBP* are a rare cause of ALS [27, 28] **OK r]** Indeed, approximately 97% of patients with ALS have features of a TDP-43 proteinopathy, with depletion of TDP-43 in the nucleus, but the formation of cytoplasmic aggregates with skein-like or compact morphology in residual motor

neurons (Figure 2A). In specific subtypes of ALS, other types of protein aggregates might be seen, such as P62-positive, TDP-43 negative protein inclusions that are caused by dipeptide repeat proteins and might be seen outside the motor system in patients with **'ALS associated with C9ORF72 mutations OK]** (Figure 2C) and neurofilamentous hyaline conglomerate inclusions (Figure 2B) and the accumulation of misfolded superoxide dismutase (SOD1) in patients with SOD1-ALS **YES]** . **[Au: green text mvoed here from the 'impaired protein homeostasis' section for flow OK]** Although protein aggregates are the hallmark of ALS, the high molecular weight **YES]** complexes that precede the formation of the aggregates, rather than the aggregates themselves[29, 30], might be the toxic species. Shedding of higher molecular protein complexes might mediate cell to cell propagation of disease, linking the progression of ALS to a prion-like mechanism, as has also been suggested for tau and synuclein-mediated diseases [31, 32].

The gross pathological features of ALS comprise skeletal muscle atrophy, atrophy of the motor cortex and pallor **LEAVE PALLOR –(more accurate in neuropathology)]** and sclerosis of the corticospinal and corticobulbar tracts **OK]**), together with thinning of the hypoglossal nerves (which are involved in the control of the muscles of the tongue) and the ventral roots of the spinal cord. Microscopic examination usually reveals a depletion of at least 50% of spinal motor neurons and diffuse astrocytic gliosis and microglial infiltration in the grey and white matter of the spinal cord (Figure 2D AND 2F). **OK OK NOW]**. Axonal loss, gliosis and myelin pallor are seen in the corticospinal tracts, and astrocytic gliosis is usually observed in the motor cortex, together with variable depletion of upper motor neurons. Skeletal muscle shows features of denervation and reinnervation, with fibre type grouping and clusters of angular atrophic fibres.

[H2] Overview of pathophysiology OK

Progress has been made in the identification of the genetic causes of ALS[21, 22] and models in rat, mouse, zebrafish, flies, worms and yeast have been developed to study the mechanisms by which gene mutations cause motor neuron degeneration and to model particular biological processes thought to be important in disease pathobiology. All of these models have limitations and none fully recapitulates human disease, which is partly because most models are based on gene overexpression (with multiple copies of the human variant inserted into the transgenic model) and because the human neuro-axis differs substantially. **OK]** from that of lower animals. **OK]** Nevertheless, findings from animal models

OK] can contribute to our understanding of the cell biology underlying neurodegeneration and can open new avenues towards targeted drug development. In reality, the cellular disruption **?OK]** in ALS is likely the result of many different interacting mechanisms that culminate in larger network disruption, and the separation of different mechanisms is somewhat artificial. **[OK]** This is exemplified by the finding that multiple factors can contribute to neuronal damage in models of *Sod1* **OK MODIFIED BY PJS ?]** mutations (Table 1). The relative extent by which each of these factors contributes to the overall pathobiology of human disease cannot be fully ascertained, it would be erroneous to assume that all of these factors are involved in all cases of ALS, as human disease is heterogeneous. Notwithstanding, each of the thematic areas should be considered in detail, as they represent our current knowledge base of the pathophysiology of ALS, and are the drivers of current and future therapeutic initiatives (Figure 3).

[H2] Impaired protein homeostasis

[] OK

Mutations in some genes **OK]** lead to the translation of proteins that are misfolded, have an abnormal cellular localization or are aberrantly formed, and that can directly or indirectly impair the proteasome or autophagy machinery of the cell, leading to impaired cellular protein turnover. Indeed, genes associated with familial ALS encode proteins that can **[OK]** promote dysfunction of the ubiquitin-proteasome system. For example, mutant SOD1 is associated with reduced expression of ubiquitin-proteasome system components [33], valosin-containing protein (VCP) and ubiquilin-2 are involved in substrate delivery to the proteasome, and this function is disrupted in the presence of ALS-associated mutations **[n SENTENCE IS OK AS IT STANDS]** [34-36]. In addition, dysregulation of chaperone proteins has been identified in ALS associated with *SOD1* and *TARDBP* mutations [37-40] . Mutations in *VAPB* (encoding vesicle-associated membrane protein associated protein B **[Au OK:]**) can cause defective activation of the unfolded protein response in disease models [41, 42].

C9orf72 **[? PROTEIN YES]** is a key regulator of autophagy initiation [43] and loss of this function might contribute to the presence of ubiquitin and p62 positive, TDP-43 negative inclusions in extra-motor areas of the central nervous system (CNS) in *C9orf72*-related ALS **[*YES*]** . **OK]** Sequestosome-1, optineurin and ubiquilin-2 have a role in the early steps of autophagy [44-46], and alsin, polyphosphoinositide phosphatase (FIG4), transitional endoplasmic reticulum ATPase (VCP) and charged multivesicular body protein 2b (CHMP2B) have roles in the maturation of autophagosomes into autophagolysosomes by regulating the fusion of autophagosomes with multivesicular bodies,

endosomes and lysosomes lysosomes [47-51]. Mutations in *SQSRM1* **[? OK]** might disrupt the correct delivery of autophagic substrates to the autophagosome [52] and mutations in *UBQLN2* and *OPTN* **OK ?]** (which both encode autophagy receptors) are also associated with ALS. The activities of sequestosome-1 and optineurin are regulated by serine/threonine-protein kinase **OK]** (TBK1) and [53, 54] haploinsufficiency of *TBK1* **[**YES is a cause of familial ALS, which supports the hypothesis that reduced substrate delivery to autophagosomes might contribute to motor neuron injury in ALS. Reduced VCP activity **YES]** has been shown to decrease the maturation of autophagosomes. Other proteins implicated in ALS pathophysiology, including alsin and FIG4 **YES ?]** , can affect autophagy at the stage of initiation, **OK ?]** although the mechanism for this is unclear[47, 55]. Both SOD1 and TDP-43 are known substrates of autophagy, suggesting that defective autophagy could contribute to the toxic accumulation of these proteins in ALS. The formation of dipeptide repeat proteins through repeat-associated non-ATG (RAN) translation from the expanded RNA repeat of the C9orf72 **[Au: this is quite technical - can we edit to this to '*C9orf72* repeat expansions might cause dysproteostasis, but this remains..' for non-experts WOULD PREFER TO KEEP ORIGINAL TEXT IF POSSIBLE ?]** gene might also result in dysproteostasis, but this remains to be conclusively demonstrated and the mechanism elucidated.

[OK

[H2] Aberrant RNA metabolism

Alteration of mRNA processing is a key theme in ALS pathogenesis[56]. **[OK]** mRNA undergoes a complex system of processing as it transits from the nucleus to cytoplasm, where it is translated into protein. In neurons, mRNAs can be transported to allow local translation in the axonal compartment. Although the functional consequences of RNA dysregulation that lead to age-related and selective degeneration of neuronal populations **NO- other neurons also affected]** remain poorly understood **[Both actually but the latter in this context** , analysis of the translatome of actively transcribing mRNAs will be essential in elucidating the upstream molecular events contributing to neuronal injury.

OK The discovery of mutations in *TARDBP* and *FUS* as rare causes of **[Au: familial? NO- ok at stands** ALS has identified a crucial pathogenetic role for RNA binding proteins that contain low complexity domains [57]. Mutant TDP-43 or FUS proteins mislocalize from the nuclear to the cytoplasmic compartment and this is hypothesised to **[OK]** result in the loss of the normal processing of their target RNAs [58, 59]. Indeed, up to one third of the transcriptome is altered in models of TARDBP-related ALS **[*OK* ?]** [60], and dysregulation

of gene expression has also been observed in relation to mutations in *C9orf72*, *SOD1*, and *FUS* **OK ?]** [61], including transcription, alternative splicing of mRNA, axonal transport of mRNAs and biogenesis of microRNAs [62, 63].

[

The GGGGCC repeat expansion in the noncoding region of *C9orf72* **[YES]** forms stable parallel uni- and multimeric G-quadruplex structures, which avidly interact with RNA processing factors [64, 65]. **[OK]** In addition, the repeat expansion gives rise to abnormal RNA species that can be identified as nuclear RNA foci and the *C9orf72* mutation **[? MUTATION OK]** might induce direct RNA toxicity, by, for example, sequestering RNA binding proteins [66-68] . Indeed, a large set **[No need to change – large set ok ?]** of proteins that bind to the expanded repeat have been identified [69]. In addition, repeat expansions could lead to the formation of R-loops **OK]** (that is, DNA-RNA hybrid structures) that increase susceptibility to DNA damage and genome instability [70, 71]. Indeed, R-Loops and genome instability due to double strand DNA breaks and defective serine-protein kinase ATM-mediated DNA repair have been identified as important components of neuronal injury due to GGGGCC repeat expansion in C9orf72 **[OK]** [72] .

OK?OK] Mutations in *ANG* (encoding angiogenin, which has a role in RNA processing [73, 74]) and *SETX* (encoding senataxin, which regulates the transcription of ribosomal RNA [75, 76]) **OK]** are associated with ALS, and might lead to disturbances in RNA metabolism. In addition, mutations in **[OK? OK]** *ELP3* (encoding elongator protein 3), *TAF15* (encoding TATA-binding protein-associated factor 2N**]OK**) and *EWSR1* (encoding RNA-binding protein EWS **OK**) [77-79] have also been associated with ALS. These genes encode proteins that are involved in regulation of RNA metabolism; ELP3 contributes to the regulation of transcription elongation, and TAF15 and EWSR1, which are functionally and structurally related to FUS, have a role in the control of transcription and alternative splicing [80, 81].

Mutations in other genes involved in RNA metabolism **[Au:** such as *TAF15*, *EWSR1*, *hnRNPA1*, *hnRNPA2B1* **[Au: This gene doesn't show up on the HUGO database, does this have another name? CORRECTED]** and *MATR3* have been implicated in ALS [82, 83] **[Au: Changed 'have been found' to 'implicated in ALS', OK? OK Please cite fewer refs here REFS REDUCED]**. The mislocalization of the mutant proteins into the cytoplasm might result in a toxic gain-of-function, and the effect of these proteins on the formation of stress granules is an area of intense research effort **[Au: why specifically on stress granules? Do the aforementioned proteins all compose stress granules, for example? PLEASE**

LEAVE AS ORIGINAL – STRESS GRANULES ARE IMPORTANT AS MOTOR NEURON INJURY IS OCCURRING] [84-86] .

[H2] Nucleocytoplasmic and endosomal transport

In addition to altering RNA metabolism **[OK]** , the GGGGCC repeat expansion in *C9orf72* is believed to alter the intracellular localisation of C9orf72 mRNA. Dipeptide repeat proteins are generated from the repeat expansion in *C9orf72* and interfere with proper nucleocytoplasmic transport and trigger neurotoxicity via several mechanisms [87, 88]. **[Au: I've deleted the sentence discussing liquid phase separation as this is quite technical. Please restrict the number of reference here to 1-2ONE OF THE REVIEWERS SPECIFICALLY ASKED FOR INCLUSION OF DISCUSSION OF LIQID PHASE SEPARATION. ONE REF REMOVED]** OK . For example, arginine-rich dipeptide repeat proteins isolated from **[OK]** *C9orf72* expansions can induce phase separation of proteins that have a role in RNA and stress granule metabolism, and produce spontaneous stress granule assembly [89]. In addition, increased binding of mRNA export adaptors to expanded C9orf72 pre-mRNAs might target those pre-mRNAs for nuclear export, which could allow RNA translation to occur with potential toxicity from the expression of abnormal dipeptide repeat protein species **YES]** [68, 90]. Indeed, sequestration of the nuclear export adaptor serine/arginine-rich splicing factor 1 (SRSF1) by the repeat expansion region of the **[Au:OK? OK]** RNA, triggers nuclear RNA export factor 1 **[?OK]** (NXF1)-dependent nuclear export of C9orf72 transcripts retaining the hexanucleotide repeats, allowing RAN translation to dipeptide repeats in the cytoplasm **[**YES . Depletion of SRSF1 in cellular and *in vivo* models reduces the production of dipeptide repeat proteins and neurotoxicity [91].

[H2] Endosomal and vesicle transport

[OK] TDP-43 is involved in the regulation of endosomal trafficking and TDP-43 loss-of-function **[PROBABLY]** has been shown to alter dendritic endosomes **[**, which resulted in reduced **signalling of neurotrophins [OK]** and detrimental effects on neuronal health [92]. Mutations in *ALS2* (encoding alsin) and *UNC13A* can alter endosomal and vesicle transport . Indeed, alsin is a guanine nucleotide exchange factor for the small GTPase Rab5, and is involved in endosome trafficking and fusion [55, 93]. UNC-13 homolog A **[**OK encoded by *UNC13A*, which is a risk factor for ALS), is involved in synaptic-vesicle priming and neurotransmitter release [94].

[H2] Axon structure and function

The finding of *DCTN* (encoding dynactin) **[YES]** , *PFN1* (encoding profilin 1) and *TUBA4A* (encoding tubulin alpha-4A chain) mutations suggests that abnormalities of proteins that are essential for axonal transport are associated with ALS [95-97]. In addition, mutations in *NEFH* (**[Au: please complete this with the gene name(s) - should this be *NEFH* and *NEFL*? YES NEFH This isn't mentioned in figure 3, should this be added here under the 'axonopathy' heading? YES]** encoding neurofilament) have also been described in a small number of patients [98], although whether these mutations are pathogenetic through axonal dysfunction remains to be seen. Rare mutations in *PRPH* encoding peripherin, another cytoskeletal protein, have been suggested to have a role in ALS pathogenesis, possibly through effects on neurofilament housekeeping including protein cargo trafficking **[Au: have mutations in *PRPH* been identified in patients with ALS? THE SENTENCE HAS BEEN ALTERED]** [99, 100].

[H2] DNA repair

Impaired DNA repair was suggested to have a role in ALS pathophysiology following the identification of *FUS* mutations, although the exact role of DNA repair failure in ALS remains to be clarified[101, 102]. Mutations in *NEK1* and *C21orf2* **[**, both of which encode proteins involved in DNA repair, have recently been identified as causes for ALS [103-105] although the biological pathways associated with their their causal role awaits confirmation **[LEAVE THIS SENTENCE AS MODIFIED HERE**

[H2] Excitotoxicity

Motor neurons are very sensitive to toxicity induced by calcium entry following excessive glutamate stimulation as they have a lower calcium buffering capacity than other neuronal subtypes and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that are more calcium permeable (as they contain less of the GluR2 subunit) [106]. In addition, excitatory amino acid transporter 2 (EAAT2), an astroglial **[**protein that is the main synaptic glutamate re-uptake transporter, is impaired in ALS, which is likely to result in synaptic glutamate abundance and motor neuron toxicity. The loss of EAAT2 has been observed in both rodent models and patients with familial or sporadic ALS. Excitotoxicity is thought to be a mechanism common to all forms of ALS, although the evidence for this remains indirect. One argument is that riluzole, which can attenuate disease progression and is an approved drug for neuroprotection in ALS, can inhibit glutamate release [107, 108]. However, whether this underlies the therapeutic effect of riluzole remains unclear.

[H2] Oligodendrocyte degeneration

Oligodendrocyte degeneration has been observed in ALS. In the healthy CNS, oligodendrocytes are replaced by the proliferation **[Au: and presumably differentiation NOT FULLY DIFFERENTIATED, so BEST TO LEAT TEXT AS IS ?]** of oligodendrocyte precursor cells, which are abundantly present [109, 110] . At least in animal models of ALS, and for reasons that are now clear **[Au: please expand on this in 1-2 sentences; what causes this failure to differentiate?] CAUSES ARE NOT KNOWN** , oligodendrocyte precursor cells **[Au: I've specified precursor cells here, OK?]OK** fail to go through the final stages of differentiation. Oligodendrocytes provide vital metabolic support to axons through the shuttling of lactate through monocarboxylate transporter 2 [111, 112], and accordingly, dysfunction of oligodendrocytes contributes to the motor axonal failure **[YES]** in ALS. Restoring oligodendrocytic function by transgenically deleting mutant SOD1 from these cells significantly slows disease progression and prolongs their life span [113]. In patients with ALS, abnormalities in oligodendrocytes can occur, but whether these changes contribute to the disease remains to be demonstrated.

[H2] Neuroinflammation

[OK Neuroinflammation can be observed in imaging studies in patients with ALS, human postmortem samples and rodent models of ALS [114, 115]. **[Au: cite fewer refs here? Refs removed].** Astrocytes and microglial cells release a number of hazardous and possibly neuroprotective factors. Deleting mutant *Sod1* ***OK]*** from these cells in a mouse model increases survival and slows disease progression [116], indicating that inflammation is an important factor for amplifying neuronal injury and disease progression in ALS. **[**OK Microglia have dual activation phenotypes, which can be neuroprotective (the M2 phenotype) or toxic (also known as classically activated, or M1 phenotype); evidence from SOD1-transgenic mice suggests the phenotype of microglia evolves with disease progression, from a neuroprotective phenotype at disease onset to a neurotoxic phenotype, with an altered cytokine release profile, at end-stage disease [117] **OK** In addition, evidence highlights complex signalling between CNS resident immune cells and peripheral cells, including monocytes and T-lymphocytes.

[H2] Mitochondrial dysfunction

Mitochondrial function is impaired in ALS and changes in mitochondrial morphology have been shown in some patients, and in the SOD1 mouse model [118, 119]. In the SOD1 model, vacuoles containing protein aggregates containing mutant SOD1 can be observed in the mitochondrial inter-membrane space, leading to impairment of protein import [120]. In addition, oxidative damage to mitochondrial proteins leads to defects in respiratory chain function in patients with ALS and in SOD1 mouse models [121], and

various experimental models of ALS have defects in axonal transport of mitochondria, which could contribute to the axonopathy at the neuromuscular junction [122, 123].

Many of the functions disrupted in ALS are regulated by signalling between the endoplasmic reticulum and mitochondria, underpinned by tight junction associations mediated by the endoplasmic reticulum protein VAPB and the outer mitochondrial protein regulator of microtubule dynamics protein [124]. These associations are perturbed by *TARDBP* and *FUS* mutations[125, 126]. TDP-43 preferentially binds to mRNAs encoding respiratory chain complex 1 subunits and causes complex 1 disassembly [127] and accumulates in the mitochondria of patients with ALS and mutations in *TARDBP* increase the mitochondrial localization of TDP-43. Suppression of TDP-43 localization to mitochondria improves mitochondrial dysfunction and reduces neuronal loss in mTDP-43 cell based models. In C9orf72-related ALS models, the dipeptide repeat protein poly(GR) appears to compromise mitochondrial function and causes oxidative stress and DNA damage [128]. *CHCHD10* mutations, which are associated with familial ALS, can promote the loss of mitochondrial cristae junctions, impair mitochondrial genome maintenance and interfere with apoptosis by preventing of cytochrome-C release [129].

[H2] Final common pathway

The main mechanism involved in the pathogenesis of ALS is probably dependent on the initial cause, although multiple mechanisms appear to explain the toxicity of one mutation and these mechanisms are likely highly interlinked. This is clearly the case for *SOD1* mutations. In the case of C9orf72 repeat expansions, multiple factors likely contribute to neuronal injury including toxic gains-of-function related to RNA foci and the presence of dipeptide repeat proteins, but loss of the normal function of the C9orf72 protein might also have a role.

Whatever the mechanisms of ALS, the end result is that the motor neuron cannot maintain its axonal projections, leading to axonal retraction and denervation of the target cell. For lower motor neurons, this results in denervation of the muscle, but for upper motor neurons results in the loss of proper control of lower motor neurons, hypertonicity and weakness .. In addition, a loss of important neural networks within motor and extra-motor domains is also apparent [130]. **[]OK** As many of the proteins encoded by genes that are implicated in ALS are ubiquitously expressed (Table 1), it is unclear why motor neurons are the most susceptible to the hazardous effects of these mutations. The large size of motor neurons, and in particular the need to maintain their long axonal projections, could make these

cells more sensitive to metabolic abnormalities than others, but other neuronal subtypes, such as sensory neurons, have even larger axonal projections. Other factors that have been suggested to have a role are the high expression of EphA4 and matrix metalloprotein 9 and the low expression of osteopontin and insulin-like growth factor 2 by motor neurons, which might limit axonal sprouting and repair. Of particular interest is that within the motor neuron pool, neurons that establish the fast fatiguable motor units die first in ALS [131, 132] , but how this relates to the other vulnerability factors needs to be clarified.

[H1] Diagnosis, screening and prevention

[H2] Clinical presentations

[OK The clinical hallmark of ALS is the involvement of both upper and lower motor neurons (Figure 1). Patients can present with symptoms of an upper motor neuron predominant onset (that is, spasticity and **[Au: muscle]** weakness) in whom lower motor neuron involvement only becomes evident at later stages of disease. [7, 133-136] **[Au: cite fewer refs here? Keep these refs if possible]** . Conversely, patients can present with symptoms of lower motor neuron dysfunction, which includes fasciculations, cramps and muscle wasting. Approximately one third of patients with ALS present with bulbar-onset disease, which is characterized by progressive dysarthria, followed by difficulty swallowing and often with associated emotional lability. Limb onset disease accounts for 60% of cases, is usually asymmetrical in presentation and can first develop in the upper or lower limb. [Up to 5% of patients present with respiratory problems and are often seen first in cardiology and pulmonology clinics prior to their referral to neurology clinics [137]. In these cases, patients can also present with unexplained weight loss. Evidence suggests that some patients with ALS are hypermetabolic; [138] although the pathophysiology underpinning this is not well understood. Cardiovascular risk factors (such as hyperlipidemia or obesity) might attenuate risk [138], but do not alter clinical outcome [139] . Patients can present with a pure motor phenotype of ALS, and have normal cognition and behaviour, but some patients can present with a purely cognitive or behavioural phenotype consistent with frontotemporal dementia(FTD)), or a mixed phenotype with minor changes in executive impairment that progress over time. Frontotemporal dementia is part of the presenting features of 13% of incident cases [2-4] and approximately 30% of all incident patients have some evidence of executive dysfunction at the time of first presentation [3, 140]. Depending on the population and the extent of cognitive testing performed, most studies have

suggested that up to 50% of patients can remain cognitively normal throughout the course of the disease [3] Behavioural changes are common in patients with ALS, with apathy as the most prevalent symptom. Detailed examination of behavioural changes in patients with ALS, using a disease specific behavioural scale (that is, the Beaumont Behavioural Index) suggests that up to 40% of incident cases have new behavioural changes that can be clustered into at least 5 different groups which roughly map to known neuroanatomical networks and pathways [141]. Substantial autonomic impairment (such as cardiovascular, gastrointestinal and bladder dysfunction) does not occur in the majority of patients with ALS.

[H2] Diagnostic criteria

No definitive test for the diagnosis of ALS is available, and diagnosis is a process of clinical investigation to exclude other possible causes of the presenting symptoms, combined with evidence of disease progression. However, the growing understanding of the extra-motor features of ALS, the presence of phenotypic overlap with other neurodegenerative diseases and the identification of genetic and pathological subtypes of ALS can confound accurate and timely diagnosis [7].

Diagnosing ALS is based on the El Escorial criteria (Box 2) [142]. Diagnosis according to these criteria requires a history of progressive weakness spreading within a region or to other regions (such as bulbar regions (speech and swallowing), cervical regions (upper limbs), thoracic regions (chest wall and abdominal muscles) or lumbar regions (lower limbs), with evidence of lower motor neuron (through the presence of specific symptoms or evidence of denervation on electromyography) and upper motor neuron (through the presence of specific symptoms and brisk deep tendon reflexes) involvement In the original criteria, diagnostic certainty ranged from Suspected ALS, (although this is no longer included in the revised criteria), to Definite ALS (in which three body regions with mixed upper and lower motor neuron findings were observed), which relates to the burden of disease. Neurophysiological findings have been classified using the Awaji Criteria, which can enhance diagnostic and prognostic sensitivity [143]. Variants of the El Escorial criteria are used in research settings and for the purposes of clinical trial enrolment, but these criteria should not be routinely used in clinical practice for routine patient management, as “possible ALS” described by the criteria is almost always ALS clinically [144, 145]. Genetic testing can also be included in patients with a strong family history of ALS [146] and clinical evidence of disease, although this is not uniformly applied across centres [147].

[H2] Cognitive and behavioural deficits

Standard diagnostic and stratification parameters for ALS do not yet include cognitive or behavioural status, which is altered in up to 50% of cases (depending on the extent of cognitive and behavioural assessment [2-4]. Various screening tools have been designed to identify patients with ALS and cognitive and behavioural changes in the clinic, such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), which is validated in several languages and is widely used, as it has a high degree of sensitivity with lower degrees of specificity [148]. Individuals with abnormal ECAS scores (after adjustment to population-based and educational norms) should be referred for a full neuropsychological evaluation [149]. The detection of cognitive and behavioural changes is important for patients with ALS and their caregivers, as executive impairment is associated with a more-rapid disease trajectory and behavioural changes are associated with higher caregiver burden [150].

[H2] Biomarkers

As ALS is a clinical syndrome with a heterogeneous phenotypic manifestation [and clinical course, diagnostic and prognostic biomarkers are urgently required for the purposes of stratification. Levels of neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain in the cerebrospinal fluid (CSF) can differentiate patients with ALS from those with mimics including cervical myelopathy, multifocal motor neuropathy and inclusion body myositis, with moderate sensitivity and specificity **[Au: could you quote some values here? 'moderate' is quite an unspecific term]**, and levels have a moderate correlation with disease progression **[Au: how do they correlate? Do levels increase with progression?]** [151-153]. However, CSF neurofilament levels are not integrated into standard clinical practice. Levels of NfL in serum are sensitive and specific for separating patients with ALS from healthy controls, but data on comparison with ALS mimics are not available.

MRI studies of patients with ALS have shown corticospinal tract degeneration, with extensive involvement within the frontal and temporal regions and basal ganglia, compared with controls Evidence suggests that selective network vulnerability of structural and functional 'connectomes' could drive the clinical manifestations of ALS, such as vulnerability of the corticospinal, orbitofrontal, orbitotemporal and frontostriatal circuits [154-156]. The presence of network disruption is also supported by findings using spectral electroencephalogram [130], and that patients with different degrees of cognitive impairment show significantly different patterns of frontal lobe metabolic impairment on ^{18}F fluorodeoxyglucose PET imaging [157]. However, neither imaging nor spectral electroencephalogram can

provide individualised data that can be used as a reliable biomarker of upper motor neuron dysfunction and of cognitive impairment in patients with ALS.

[H2] Differential diagnosis

The differential diagnosis in patients with pure bulbar pure upper motor neuron or pure lower motor neuron presentations includes ALS variants, treatable ALS mimics and disorders with a more benign prognosis [134, 158]. Other forms of motor neuron disease include progressive muscular atrophy (that is, the exclusive degeneration of lower motor neurons) and primary lateral sclerosis (that is, the exclusive degeneration of upper motor neurons). Some patients with progressive muscular atrophy have mutations in genes associated with ALS[159]. Similarly, patients with primary lateral sclerosis may have a family member with ALS and most autopsies of patients with primary lateral sclerosis show subtle signs of ALS pathology in the lower motor neurons within the brain stem and spinal cord [135, 158].

Several conditions have similar initial clinical features as ALS and should be considered in the differential diagnosis [145], including cervical myelopathy, multifocal motor neuropathy, myasthenia gravis, Lambert Eaton myasthenic syndrome and inclusion body myositis. Features that should alert the clinician to a possible mimic syndrome include presentation with of symmetrical findings; prominent extensor plantar responses (which should raise suspicion of a cervical myelopathy) and the presence of sensory findings. Although sensory symptoms are common in ALS, clinical evidence of sensory loss is atypical and should trigger further investigations. In addition, the presence of substantial weakness in the absence of wasting – which is common in multifocal motor neuropathy and myasthenia gravis – and the presence of disproportionate involvement of quadriceps – which is common in inclusion body myositis – may indicate the presence of an ALS mimic syndrome [160]. As ALS is a progressive disease, failure of the condition to progress over months should also trigger a re-investigation [161].

[H2] Staging and prognosis [

Several different staging systems for ALS have been described (Figure 4) [162-165], including the King's system, which is based on the number of affected regions of the body, and the Milano-Torino system (MITOS), which is based on a clinical scale The prognosis of ALS is highly variable and prognostic algorithms have been generated from population-based and clinical trial-based datasets [166, 167]. Negative prognostic indicators include bulbar or respiratory onset disease, the presence of executive impairment or frontotemporal dementia and weight loss. Several biochemical markers of prognosis have been

reported including serum urate, serum creatinine, serum chloride, and increased serum and CSF neurofilament levels [153, 168-170] **[Au: please cite fewer refs here. Keep if possible?]** Declining respiratory function, measured by slow vital capacity, forced vital capacity and sniff nasal inspiratory pressure also correlate with short survival [166, 167, 171, 172]. **[Au: please limit the number of references here to 1-2. Keep if possible?]**

[H2] Clinical genetics and predictive testing

Consensus guidelines recommend genetic testing of probands with ALS who have a first or second degree relative with ALS and/or frontotemporal dementia [19, 173]. As the genetic risk for ALS depends on ancestral origin, the genetic testing should be contextualized; for example, *C9orf72* variants are rare in Asia, whereas mutations in *OPTN* are more common in Asian than in European populations. Although the potential benefits of genetic testing for patients are clear and could improve knowledge about their disease, family planning and their possible inclusion in clinical trials, individuals also have a right not to know their genetic status. Pre-symptomatic testing of family members of patients with ALS remains controversial. Guidelines for genetic testing in research settings have been published [174], but most centres do not advocate routine testing outside of specialist centres [147].

[H1] Management

ALS management is best achieved by a multidisciplinary approach to care, comprising a clinical team with different specialities, including neurologists, psychologists, nutritionists, pulmonologists, physical therapists, speech therapists and specialized nurses[175, 176]. Multidisciplinary care increases survival [177-179], reduces the number of hospital admissions and shortens hospital stays [178] and increases quality of life of patients with ALS [180]. This is likely related to the optimization of pharmacological and non-pharmacological interventions and enhanced adherence to treatment guidelines.

[H2] Disease-modifying therapies

Although > 50 drugs with different mechanisms of action have been studied for the treatment of ALS, only 2 compounds (riluzole and edaravone have come to market. The negative results of these trials might include clinical and pathogenetic heterogeneity in disease, and faults in trial design [181].

Riluzole was the first FDA approved treatment for ALS, and, although the mechanism of action is poorly understood, is speculated to reduce glutamatergic neurotransmission, by blocking voltage-gated sodium

channels on presynaptic neurons. . In the original trial, Riluzole, increased 18-month survival of patients by 3 months compared with placebo , but had no significant effect on muscle strength [182]. Riluzole is a relatively safe drug, although the most common adverse effects are an increase in liver enzymes and asthenia (that is, a lack of energy) and some cases of fatal hepatic failure and pancreatitis have been reported. In addition to the traditional tablet form of the drug, an oral suspension has been produced and marketed in some countries for patients who are unable to swallow solid forms of the drug, owing to severe dysphagia [183]. Edaravone, which is thought to act as an anti-oxidant agent has a beneficial effect on progression in a highly selected cohort of patients with early onset and rapidly progressive disease [184], and accordingly, has been licensed by the US FDA but not by the European Medicines Agency. Whether edaravone should be provided to all patients of ALS regardless of clinical presentation is a matter of debate [185]

[H2] Symptomatic treatments

Other symptoms of ALS can be treated with pharmacological and non-pharmacological interventions. Nuedexta may improve bulbar function [186] and is available in the US but not in Europe. However, most of these therapies for the symptoms of ALS have not been tested in randomized controlled trials and are based on management of other diseases.

[H3] ***Spasticity.*** Spasticity is present in most patients with ALS, but only a small proportion need treatment. The most commonly used drugs are baclofen and tizanidine (both of which are muscle relaxants) although no randomized controlled trials in patients with ALS have been conducted. When patients have severe, disabling spasticity, baclofen can be administered through an intrathecal pump. . Cannabinoids have been approved for the treatment of spasticity in patients with multiple sclerosis and are also used off-label or as a self-prescribed medication in patients with ALS[187].

[H3] ***Sialorrhoea.*** Sialorrhoea (that is hypersalivation), causing drooling and the pooling of saliva within the oral cavity is one of the most disturbing symptoms in patients with ALS, and is more commonly observed in patients with bulbar-onset disease and during late-stages. Sialorrhoea can be treated [with anticholinergic drugs, such as scopolamine, atropine, hyoscine, amitriptyline and glycopyrrolate. Adverse effects associated with the use of anti-cholinergics include blurred vision, mouth dryness and constipation, and these drugs are contraindicated in patients with heart conduction disturbances and prostatic hypertrophy. In patients in whom pharmacological treatments are ineffective or are not

indicated, botulinum toxin A or B injections into the salivary glands can used to treat sialorrhoea[188, 189]. Salivary gland irradiation has been also proposed [190].

[H3] Pain. Pain is reported in 15–85% of patients with ALS, depending on the duration of the disease and the setting of the study, and is more frequently of nociceptive than of neuropathic origin. [191] Depending of the type of pain, pharmacological treatments include gabapentin, pregabalin and tricyclic antidepressants (for neuropathic pain), and NSAIDs, opioids and cannabis for nociceptive pain), but no randomized controlled trials evaluating treatment of pain in patients with ALS are available. Nociceptive pain can be also treated with intra-joint injections of lidocaine or steroids, and physical therapy, including assistive range-of-motion exercises.

[H3] Muscle *cramps.* Muscle cramps are the main cause of pain in about one-quarter of patients with ALS (mainly patients with the spinal onset disease) and are caused by the instability of motor units [192]. Commonly used treatments for muscle cramps include quinine sulphate, levetiracetam and mexiletine. **I**ndeed, mexiletine has been shown to induce a significant dose-dependent reduction in muscle cramps in a phase 2 randomized controlled trial in patients with ALS [193]. Of note, the FDA has advised against the use of quinine sulphate for the treatment of cramps because it can cause cardiac arrhythmias, bradycardia and prolongation of Q-T interval.

[H3] ***Dysphagia***

Dysphagia is reported by about 60% of patients with spinal onset ALS, within two years from onset and 100 % of patients with bulbar-onset disease [194]. Several strategies can be implemented to reduce the effects of dysphagia in patients, including dietary changes such as modification of the consistency of the diet, the use of fluid thickeners and prescription of high-protein and high-caloric supplements, swallowing facilitating manoeuvers and exercises (such as oral and pharyngeal range-of-motion exercises, head postures and the technique of supraglottic swallow). An option for severe difficulties with swallowing is to use enteral nutrition via the insertion of a gastrostomy tube. No established criteria are available for the initiation of enteral nutrition in patients with ALS, but weight loss of >5% or unsafe swallowing are generally considered to be red flags that should prompt intervention. [175]. Several techniques are available for minimally invasive tube insertion and open surgery is not recommended [195, 196]. Parenteral nutrition provided through a central venous catheter is an alternative to enteral nutrition

in patients with ALS who have severe respiratory insufficiency for whom PEG **[Au: Percutaneous endoscopic gastrostomy]** or RIG **[Au: Radiologically Inserted Gastrostomy?** are contraindicated [197, 198].

[H3] ***Dysarthria.*** Dysarthria is the presenting symptom in 30% of patients and is found in > 80% of patients during the course of the disease, up to complete anarthria. Speech therapy can delay the progression of dysarthria and augmentative-alternative communication **[**techniques such as customised software are the treatment of choice and can enhance quality of life in the most advanced phases of ALS [199]. Communication techniques based on brain-computer interfaces (BEST LEAVE THIS IN PLACE] have been developed, but their use in the clinical setting is still very limited as their effectiveness has not been definitely demonstrated [200]. Moreover, the use of brain-computer interfaces might be hindered by patients' cognitive dysfunction or old age [201].

[H3] ***Deep venous thrombosis.*** Patients with ALS have leg weakness and reduced mobility, which can increase the risk of symptomatic and asymptomatic deep venous thrombosis (DVT). The annual incidence of DVT in patients with ALS ranges from 2.7 to 11.2% [202, 203]. In the absence of specific studies on the prevention and treatment of DVT in ALS general guidelines should be applied, including the use of compression stockings and anticoagulation therapies

[H3] Mood alterations. Depression is a relatively common symptom in patients with ALS and has been found in up to 50% of patients. Depression is generally treated with selective serotonin reuptake inhibitors (SSRI) or tricyclic antidepressants. Pseudobulbar affect (that is, episodes of uncontrollable crying or laughing) is a distressing symptom that has been reported in up to 50% of patients with ALS [204] **[**and can be treated with SSRIs and tricyclic antidepressants, although this is off-label. Dextromethorphan (a sigma-1-receptor agonist and an uncompetitive NMDA receptor antagonist) and low-dose quinidine were effective in reducing symptoms of pseudobulbar affect by 50% in patients with ALS or those with multiple sclerosis [205].

[H3] Cognitive impairment. Cognitive impairment, in particular frontotemporal dementia, is one of the most disabling symptoms in patients with ALS. No pharmacological therapy is effective for the treatment of frontotemporal dementia, and acetylcholinesterase inhibitors, which are used for Alzheimer disease, are not effective. However, some symptoms of frontotemporal dementia can be pharmacologically treated; evidence suggests SSRIs might help to control the loss of inhibition, overeating and compulsive

behaviour, and antipsychotics can be used to reduce restlessness. Education of caregivers about the symptoms of frontotemporal dementia can be useful to help the management of patients at home [206].

[H3] Respiratory insufficiency.

The vast majority of patients with ALS die from respiratory failure. Non-invasive ventilation is the symptomatic treatment of choice for respiratory failure, and provides significantly longer survival compared to those who do not use NIV (316 vs 229 days) and improves quality of life [207] [208]. Accepted criteria for starting non-invasive ventilation are symptoms or signs related to respiratory muscle weakness (such as, dyspnoea, orthopnoea or daytime fatigue), a vital capacity of < 80% of predicted levels, $PaCo_2$ > 45 mmHg, SaO_2 < 90% during ≥ 5% of sleep time [176]. One distressing symptom that is related to respiratory muscle weakness in patients with ALS is the inability to cough effectively. This can be controlled by the use of cough-assist devices, such as the breath-stacking technique or a mechanical insufflator-exsufflator [209].

[H2] End of Life Management

The end of life phase for patients with ALS can be difficult to define, although recent staging systems including KINGS and MITOS [are useful in this regard. The end of life period can be particularly challenging and is characterized by substantial mobility, communication and, in some cases, cognitive difficulties. An early discussion of end of life issues will ensure that patients can communicate their wishes before the onset of substantial communication and cognitive difficulties, can avoid unwanted interventions or procedures, and can provide time for reflection and the integration of choices within the patient's priorities and life plans. In addition, such discussions can alleviate patient's fears, especially around fatally choking. The attitudes, culture and personal values of patients, caregivers and health care providers can influence the timing and content of end of life discussions, decision-making and the patient's acceptance or refusal of interventions and treatment options. Some patients with ALS might choose life-prolonging measures, but others might contemplate life-limiting procedures; the availability and utilization of different interventions and technologies, such as assisted death and tracheostomy, varies across centres and between countries. Advance care directives are recognized as important at end of life in ALS, and provide patients with the option to exercise autonomy regarding preferred end of life management strategies. Formal care at the end of life should aim to maximize quality of life of both the patient and caregiver and, where possible, incorporate appropriate multidisciplinary care including palliative care options.

[H1] Quality of Life

Much of the effort of physicians and other health care providers is focused on optimizing the quality of life (QOL) of patients with ALS. **[Au: green text moved to here for flow. OK]** The choice of a specific QOL instrument is complex, and has been reviewed [210]. The perception by individuals with ALS of their QOL takes shape at the time of disclosing the diagnosis, and can be influenced by the manner in which they are informed . Well-recognized systematic approaches are available, such as the SPIKES approach, that can convey the diagnosis in a less distressing manner and can leave the patient feeling hopeful and supported [211-213].

[Au: I've deleted the text stating healthy individuals think the QOL of patients with an illness is lower, and that HRQOL is not the same as QOL, as this isn't needed here] Health-related QOL (HRQOL) refers to an individual's perception of their QOL as a function of physical and mental well-being [214]; measures of HRQOL generally decline as ALS advances [210, 215]. In contrast, OQOL **[Au: overall QOL?]** encompasses medical factors and a wide variety of non-medical factors, such as family, friends, occupation, financial well-being, spirituality or religion and existential concerns [216]. Patients with ALS often view their OQOL as good, which persists despite the progression of physical disability [217, 218]. This might be explained by a 'response shift' (also called a frame shift or well-being paradox), whereby the individual recalibrates the factors that are deemed meaningful to maintenance of their QOL. Most commonly, this centres around the decreased importance of physical activities and the greater role of interactive and existential factors, such as social relationships and spirituality [219-221]. However, not all patients maintain a high QOL with advancing illness. Many factors can negatively affect QOL in patients with ALS, identifying potential areas for intervention, although other factors can improve QOL (Figure 5) [180, 207, 214, 222-228] **[Au: please restrict the number of references here to 1-2]** .

Despite good QOL of patients with ALS in aggregate **[Au: '..a good QOL of most patients with ALS'? edited for brevity]** , psychological health is, on average, poorer than that of the population as a whole [229]. This has substantial implications as depression, hopelessness and anxiety all associated with a poor QOL. **[Au: I've moved the green text to here from earlier on for flow]** Psychological interventions have

been less well studied **[Au: than what? please add a comparator here]** [230] and this warrants further attention.

QOL can affect the wishes for care of patients with ALS at the end of their lives. **[Au: edited for flow]** In a study from the Netherlands, 16.8% of patients with ALS chose physician-assisted death, common reasons for which were hopelessness, loss of dignity, dependency on others and fatigue [215]. Similarly, the decision for euthanasia in patients with ALS in Washington State was driven by loss of autonomy, participation in enjoyable activities and dignity [216]. These studies do not prove poor QOL in these individuals, but they do raise this as a concern. The quality of death in patients with ALS has been studied less comprehensively **[Au: than QOL?]** . Death was perceived as peaceful by 88% to 98% of caregivers in Germany, the United Kingdom, the United States and Canada [217, 231].However, caution must be used in interpreting grouped statistics. Incompletely relieved symptoms such as coughing from **[Au: excess?]** mucus, restlessness, anxiety and muscle **[Au: added muscle here]** cramps resulted in moderate to severe suffering in the last 24 hours of life in 8 of 171 patients [217].

[H1] Outlook

The knowledge of ALS and the care of patients with this condition have increased substantially in recent years, and this trend is likely to continue. 25 years ago, riluzole had not been enrolled in a clinical trial, non-invasive ventilation was not in routine use for patients, the pathological basis of ALS as a TDP-43 proteinopathy was unknown and no genetic causes for ALS had been identified. In addition, the El Escorial criteria were not developed, no simple ALS functional scale existed, multidisciplinary care was in its infancy and the recognition of cognitive change in patients with ALS was limited, and the link with frontotemporal dementia was not made. What will be different in another 25 years, and how much of what we regard as self-evident now, will be overturned, is tempting to consider.

[H2] Epidemiology

We can expect that the numbers of patients with ALS will increase in the future [218], and that population differences in incidence and phenotype will be recognized. Better multidisciplinary care and an improved understanding of interventions means that a patient diagnosed with ALS can expect to live

longer than previously. In addition, the development of new drugs to improve respiratory function or directly affect the disease process are expected to improve survival.

[H2] Pathophysiology

A big barrier to effective ALS treatments is due to our lack of knowledge of the pathological pathways that lead to the disease, and how they affect the overall integrity of brain networks. Our understanding of ALS is improving, including contextualizing the role of TDP-43, the importance of RNA processing for motor neurons, the spread of disease and the molecular cascades that lead to neuronal death. The development of new cellular and animal models of ALS is beginning to lead to improvements in our understanding of the disease , both because the molecular pathways can be dissected more easily, and because the models can be used to more effectively to identify drugs worth enrolling into human trials. These insights are the result of genetic findings, which have led to experiments aiming to understand how loss-of normal protein function and gain-of toxic function cause ALS. As the number of genes implicated in ALS increases and laboratory models improve, we can expect to design new drugs to intervene in those pathways.

Indeed, our understanding of the genetics of ALS has transformed over the last 25 years, with the finding that both familial and sporadic ALS have a genetic basis and the number of validated involved genes steadily increasing. These findings are in large part due to the willingness of the ALS research community to collaborate, which has generated the huge datasets required for credible gene discovery. The finding that the genetic architecture of ALS includes an important role for rare genetic variation has consequences for the likelihood that gene therapy could be effective in this disease. Indeed, as rare variants are more likely to have a large effect on the risk of disease and can be directly manipulated by gene therapy, we can expect to see precision medicine spearheaded by targeted gene therapies.

The relationship between ALS and cognitive, cerebellar, autonomic and other non-motor changes is an area of research that is expected to grow. One consequence of this research is that ALS is probably primarily a disease of neural networks, which is defined by the involvement of upper and lower motor neurons, but that can also affect other cell populations and neuronal networks. We can also expect an increased understanding of the role of inflammation in ALS, both in triggering disease and influencing the rate of progression.

[H2] Diagnosis and prognosis

The use of biomarkers for ALS has been investigated for many years, although our understanding has only recently matured for research to yield useful results. Diagnostic biomarkers would be useful for individuals with an atypical or complicated presentation, biomarkers for prognosis would be useful for planning treatment options, and biomarkers of disease progression would be useful for monitoring response to existing therapies or potential new therapies in a clinical trial. New signal analysis based technologies will become available as biomarkers that can image the living human brain [139].

[H2] Management

[H3] Clinical Trials

The validity of pre-clinical studies should be evaluated rigorously by evidence-based analyses, and translation of new therapies to humans should be undertaken only if findings are robust and reproducible. Moreover, as ALS is a human disease, testing safe candidate compounds without prior testing in animal models could be undertaken. In this instance, careful phase I and 2 studies including detailed pharmacokinetic studies with extensive dose-finding and toxicity studies will be needed. As some previous ALS clinical trials failed due to faulty trial design, a detailed correlative analysis of drug levels in serum and CSF should be undertaken in early phases trials, and all trials should include a biomarker readout to confirm that the drug is reaching its target. **[Au: what do you mean by 'target engagement'? Please clarify]** .

The failure of previous clinical trials for ALS could also result from disease heterogeneity. Methods to stratify patients that have a shared pathobiology are urgently required, and in the absence of this, pre-specified, post-hoc analyses should be used to identify potential responder groups. This is exemplified by a recent successful Phase 3 trial of edaravone [184], as recruitment to this trial was based on a post-hoc analysis to identify possible responders, and stringent recruitment criteria were used to provide a clinically homogeneous population that were likely to respond to treatment.

[H3] New Drugs

An extensive pipeline of new therapeutics for ALS is available, and some of these drugs target known mutations and pathogenetic pathways. Symptomatic therapies including tirazemptiv based on improving respiratory function in patients with ALS are currently in Phase 3 trials and exciting Phase I trials assessing the use of antisense oligonucleotides in *SOD1* and *C9orf72* **[**related ALS are underway. In

the future, treatments are likely to be targeted at specific subgroups of patients and biomarkers that are personalized to the individual disease subtype and have been developed from patient subcohorts that have been extensively phenotyped and stratified using genomics, transcriptomics, metabolomics and advanced imaging and signal analysis.

Display items

Box 1. Mechanisms of SOD1 toxicity in cellular and rodent models [Au: Title OK? I have deleted this figure as this is very repetitive with figure 3 and made the figure legend into a box. We can illustrate the mechanisms included in this figure (prion-like seeding, etc.) in figure 3 if you wish, although I don't think this is necessary as this is nicely described in this box] .

Transgenic mice with mutations in *SOD1* (encoding superoxide dismutase, SOD1 **[Au: I've added the gene product here as this is useful to note]**) can be used to study ALS pathophysiology. These mice over-express mutant SOD1 and many have an aggressive disease course over approximately 80-90 days. However, they display quite well clinical and pathological features similar to human ALS.

xx **[Au: Please add 1-2 sentences here discussing the phenotype of these mice – DONE do they show sensorimotor dysfunction, for example? Reduced bowel and bladder function? I have adapted the table that was in figure 1 into continuous prose (highlighted in yellow). I've also added a reference to the NRNeuroscience review (ref 242) - please check this carefully OK]** . *SOD1* mutations can drive neurotoxicity in several ways, including protein misfolding **[Au: presumably the misfolded protein here is SOD1? BUT ALSO AGGREGATES OF NEUROFILAMANT PROTEINS],** proteasome impairment, excitotoxicity, oxidative stress, ER stress, impaired axonal transport, axonopathy, inflammation, altered RNA processing and mitochondrial dysfunction. [232] Other mechanisms of SOD1-related neurotoxicity have recently emerged and have gained interest. SOD1 can acts as a transcription factor for genes involved in **resistance to oxidative stress PLEASE LEAVE AS IS. '?]** and repair of oxidative damage **[Au: DNA repair?]** [233]. RNA oxidation is emerging as a prominent pathological outcome of generalized oxidative stress in the cell with increasing importance in neurodegeneration **[Au: does RNA oxidation occur in *SOD1* transgenic mice?YES]** . **[Au: what do you mean by this? Do you mean astrocytes and oligodendrocytes with mutations in *SOD1*?]** Astrocytes and oligodendrocytes reprogrammed from fibroblasts of patient with SOD1 mutations have been shown to induce hyperexcitability and cell death **[Au: cell death of the motor neurons only, or also of astrocytes and oligos?]** in healthy control motor neurons. Glial toxicity is mediated through both contact (lactate independent) and soluble mechanisms and is rescued by SOD1 knockdown using short hairpin RNA in glia derived from patients with AOS1-related familial ALS, but also in glia derived from patients with sporadic ALS without SOD1 mutation [113]. Wild-type and mutant SOD1 proteins form insoluble **intraneuronal** fibrils, which aggregate with increased propensity in the mutant form. A prion-like transmission of mutant SOD1 fibrils can seed wild-type SOD1 protein aggregation in neighbouring neurons and propagate neuronal injury[234].

Box 2. El Escorial criteria [Au: please add these criteria here] .

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Figure 1. Clinical manifestations of ALS [Au: Note this has been renumbered as figure 1, so the symptoms of ALS are introduced early on in the manuscript] .

Although motor manifestations such as muscle weakness and difficulty swallowing are the main clinical manifestations of amyotrophic lateral sclerosis, up to half of patients have non-motor symptoms, such as cognitive defects.

Figure 2. Histopathology of ALS.
a) **[Au: I've edited this for brevity and house style, please check this carefully]** Normal localization of TDP-43 in the nucleus (black arrow head), and aberrant localisation in a diseased neuron with loss of nuclear expression and a 'skein-like' inclusion in the cytoplasm (black arrow). b) **[Au: I've deletd the H&E image as this isn't needed here OK]** Normal motor neuron (black arrow) and a hyaline conglomerate inclusion that stains for SMI31 (black arrow head) in a patient with ALS caused by a *SOD1* mutation. c) TDP-43-negative, **p62 positive [OK]** dipeptide repeat inclusions with a 'stellate' morphology in the pyramidal cells of CA4 (black arrow) and granule cells of the dentate fascia (black arrow head) in the hippocampus of a patient with ALS caused by a mutation in *C9orf72*. d) The spinal cord ventral horn of a patient with ALS and a **[Au: I've deleted normal, healthy is sufficient here]** healthy individual (e) showing a depleted numbers of motor neurons in ALS (arrows). F) CD68 (a microglial marker) immunohistochemistry shows marked microglial **[Au: I'm not sure what you mean by this, please clarify DONE]** reactivity in the lateral tracts (black arrow) and ventral **[Au: I've changed anterior to ventral, OK? OK]** horns (black arrowhead), with no labelling **[Au:OK? OK]** in the dorsal columns (white arrow).

Figure 3. Pathophysiology of ALS [Au: figure title OK?] .

Mutations in several amyotrophic lateral sclerosis (ALS) causative genes **[Au: do you mean mutations in these genes? reworded]** can exert motor neuronal injury through more than one pathophysiological mechanism, although these mechanisms are often interlinked. *SOD1* is the longest studied gene implicated in ALS and has been linked to the most pathophysiological mechanisms, although the effects of **mutations in** *ALS3* and *ALS7* are still unknown. Aberrant RNA metabolism and impaired protein homeostasis are predominant factors linking multiple ALS causative genes **[Au: what do you mean by 'most causative genes'? Please clarify DONE]** to neuronal injury. Mitochondrial dysfunction can arise from a mutation in *CHCHD10* and from secondary respiratory chain deficiencies that arise from protein aggregates generated in the presence of other ALS genetic mutations **[Au: can we just say 'arise from protein aggregates' here? REWORDED]**. Both cases lead to an increase in oxidative stress, which puts further stress on an already impaired protein homeostasis system. Other mechanisms of ALS can directly

alter neuronal function (such as nuclear export, impaired DNA repair, dysregulated vesicle transport and axon dysfunction) and the function of non-neuronal glial cells. **[Au: I've added in the highlighted text so all pathologies illustrated in the figure are mentioned in the legend, OK? Please feel free to edit this OK]** The interplay of mechanisms is indicated by arrows.

Figure 4. Staging systems for ALS.

[Au: green text moved here from the main manuscript text for flow, and edited for brevity] The King's staging system is based on the number of body regions affected by ALS and the presence of respiratory or nutritional failure [162]. The Milano-Torino staging **[Au: definition of MITOS OK?]** (MITOS) system is based on the ALS functional rating scale (ALSFRS-R), a 48 point clinical measurement scale that records changes in bulbar, gross motor, fine motor and respiratory parameters **[Au: can we edit this to '...changes in four functional domains: bulbar, gross motor, fine motor and respiratory'? If not, what are the functional domains that are referred to in the figure?]** [163]. These staging systems do not incorporate cognitive or behavioural changes. The King's staging system is sensitive to early changes in ALS, but the sensitivity of the MITOS scale is greater in the later stages of disease [164, 165].

Figure 5. Factors affecting QOL in patients with ALS.

Several factors that positively or negatively affect overall quality of life (QOL) and health-related QOL (HRQOL) have been identified in patients with amyotrophic lateral sclerosis. These factors include motor symptoms, psychological symptoms and therapeutic interventions. AAC, augmentative and assistive communication; VC, verbal communication.

992 **Table 1. Genes implicated in ALS.**

Gene locus	Gene (protein) [Au: I've reformatted this so the gene name is first and the protein name following in brackets]	Inheritance	Implicated disease mechanisms	References
ALS1	*SOD1* (Superoxide dismutase 1)	AD/AR	Oxidative stress	235, 236
ALS2	*ALS2* (Alsin)	AR	Endosomal trafficking	237, 238
ALS3	*Unknown*	AD	Unknown	239
ALS4	*SETX* (Senataxin)	AD	RNA metabolism	240
ALS5	Unknown	AR	DNA damage repair, axon growth	241
ALS6	*FUS/TLS* (Fused in sarcoma/translated in liposarcoma)	AD/AR	RNA metabolism	242, 243
ALS7	Unknown	AD	Unknown	244
ALS8	*VAPB* (Vesicle associated membrane protein (*VAMP*) – associated protein B)**[Au: should this be split up into two rows? Have *VAMP* and *VAPB* both been implicated in ALS?]**	AD	ER stress	42
ALS9	*ANG* (Angiogenin)	AD	RNA metabolism	245
ALS10	*TARDBP* (TAR DNA binding protein)	AD	RNA metabolism	27, 246
ALS11	*FIG4* (Polyphosphoinositide 5-phosphatase **[Au: protein name OK?]CORRECTED**)	AD	Endosomal trafficking	247
ALS12	*OPTN* (Optineurin)	AD/AR	Autophagy	248
ALS13	*ATXN2* (Ataxin 2)	AD	RNA metabolism	249
ALS14	*VCP* (Valosin-containing protein)	AD	Autophagy	36
ALS15	*UBQLN2* (Ubiquilin 2)	XD	UPS, autophagy	34
ALS16	*SIGMAR1* (Sigma non-opioid intracellular receptor 1)	AD	UPS, autophagy	250, 251
ALS17	*CHMP2B* (Charged multivesicular body protein 2B)	AD	Endosomal trafficking	252
ALS18	*PFN1* (Profilin 1)	AD	Cytoskeleton	97
ALS19	*ERBB4* (V-erb-b2 avian	AD	Neuronal	253

	erythroblastic leukaemia viral oncogene homolog 4)		development	
ALS20	*HNRNPA1* (Heterogeneous nuclear ribonucleoprotein A1)	AD	RNA metabolism	82
ALS21	*MATR3* (Matrin 3)	AD	RNA metabolism	83
ALS22	*TUBA4A* (Tubulin alpha-4A) **[Au:protein name OK? corrected]**)	AD	Cytoskeleton	102
ALS-FTD1	*C9orf72* (Chromosome 9 open reading frame 72)	AD	RNA metabolism, autophagy	5, 6
ALS-FTD2	*CHCHD10* (Coiled-coil-helix-coiled-coil-helix domain containing 10)	AD	Mitochondrial maintenance	255
ALS-FTD3	*SQSTM1* (Sequestosome 1)	AD	Autophagy	256
ALS-FTD4	TBK1 (TANK-binding kinase 1)			53, 54

993 AD, autosomal dominant; AR, autosomal recessive; XD, X-linked dominant

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References **[Au: Please select ~10 references of particular importance and give a single sentence for each stating why the paper is important. Please copy the whole reference (not just the number, since this will inevitably change) to a separate list and provide the justifying sentence after it.]**

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