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1 **Amyotrophic lateral sclerosis**

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20

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40

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45

46 **Abstract** Amyotrophic lateral sclerosis (ALS), also known as Motor Neuron Disease (MND), is
47 characterized by the degeneration of both upper and lower motor neurons, leading to muscle weakness
48 and eventual paralysis. Until recently, ALS was classified primarily within the neuromuscular domain,
49 although new imaging and neuropathological data have indicated the involvement of the non-motor
50 neuraxis in disease pathology. In most patients, the mechanisms underlying development of ALS are
51 poorly understood, although a subset of patients have familial disease and carry mutations in genes that
52 have various roles in neuronal function. Two disease modifying therapies which can slow disease
53 progression, are available for the treatment of ALS, but patient management is largely mediated by the
54 use of symptomatic therapies, such as the use of muscle relaxants for spasticity and speech therapy for
55 dysarthria.

56

57

58 **[H1] Introduction**

59 Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative syndrome that is
60 characterized by the degeneration of both upper (that is, neurons that project from the cortex to the
61 brain stem and the spinal cord) and lower (that is, neurons that project from the brainstem or spinal

62 cord to the muscle) motor neurons leading to motor and extra-motor symptoms (Figure 1). The initial
63 presentation of ALS can vary between patients; some present with spinal-onset disease (that is, the
64 onset of muscle weakness of the limbs), but others can present with bulbar-onset disease (characterized
65 by dysarthria – difficulty with speech – and dysphagia – difficulty swallowing. In most patients, the cause
66 of ALS is unknown, although some individuals develop familial forms of the disease, which are
67 associated with mutations in genes that have a wide range of functions, including functions in non-
68 motor cells. In the familial forms of the disease, some of the implicated genes are incompletely
69 penetrant, and with rare exceptions, genotype does not necessarily predict phenotype ¹. Although the
70 primary symptoms of ALS are associated with motor dysfunction (such as muscle weakness, spasticity
71 and dysphagia), up to 50% of patients develop cognitive and/or behavioral impairment during the
72 course of disease and 13% of patients present with concomitant behavioral variant frontotemporal
73 dementia (bv-FTD)²⁻⁴. The high prevalence of cognitive and/or behavioural symptoms, coupled with the
74 finding of a hexanucleotide repeat expansion in *C9orf72* as the major genetic cause of ALS and FTD ^{5,6},
75 have contributed to the re-characterization of ALS as a neurodegenerative, rather than a neuromuscular
76 disorder, and have signposted the direction of research over the coming decade.

77

78 The classification of ALS can vary depending on the criteria used. The traditional definitions of ALS
79 subgroups are based on the extent of upper and lower motor neuron involvement, although other
80 classification systems include different parameters, such as the site of onset (that is, bulbar or spinal
81 onset of disease), the level of certainty of diagnosis according to the revised El Escorial Criteria and
82 heritability (sporadic or familial disease)⁷. To date, none of these classification systems have
83 incorporated the cognitive or behavioural symptoms and within each classification system a range of
84 sub-phenotypes and clinical trajectories can be demonstrated.

85

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

91

92

93 **[H1] Epidemiology**

94

95 **[H2] Descriptive epidemiology**

96 The majority of population based epidemiological studies for ALS have come from high quality European
97 patient Registers ⁸. These European population based Registers have been combined to form the
98 European ALS Epidemiology Consortium (EURALS), which has provided data comparing the incidence of
99 ALS between European countries ⁹. In Europe, the incidence ranges from 2-3 cases per 100,000
100 individuals. Defined geographical areas are ideally suited to estimate the incidence and prevalence, and
101 to support more-detailed studies of risk, clinical trajectory, outcome and utilization of services for ALS⁸.
102 As ALS is a rare disease, a population-based approach with multiple sources of ascertainment is the best
103 way to describe the entire phenotypic spectrum ¹⁰ as population-based registers provide more complete
104 information about the disease than datasets from specialist clinics, which are often biased in favour of
105 younger patients and those with less severe disease ¹⁰. Similarly, clinical trial cohorts such as those
106 collected within the US-based pooled resource open-access ALS clinical trials database (ProACT) dataset
107 also select for patients with ALS who have better prognosis; survival within these cohorts is ~12 months
108 longer than that of true population-based cohorts.

109 Contrary to earlier assumptions, the incidence of ALS has been shown to differ based on ancestral
110 origin; studies in populations of European origin have shown a crude incidence of >3 cases per 100,000
111 individuals ^{11,12}, but incidence rates are lower in East Asia (around 0.8 per 100,00) and South Asia (0.7
112 per 100,000). In some regions (such as Guam and the Kii peninsula of Japan) the reported incidence
113 was very high, but dropped substantially over the past 30 years for reasons that remain unclear. In areas
114 where different ancestral populations live in close proximity (as in Northern America), the incidence
115 rates of ALS in indigenous populations is particularly low (0.63 cases per 100,000 individuals)¹³, whereas
116 reported incidences in regions of relatively homogeneous populations (such as Ireland, Scotland and the
117 Faroe Islands) are high (2.6 cases per 100, 000 individuals) ^{9,14}.

118

119 In addition, variations in the phenotype and natural history of ALS have been reported in different
120 ancestral populations; indeed reported survival of patients with ALS is much shorter in Europe (24
121 months) than in Central Asia (48 months) ¹⁵. In addition, admixed populations (that is, populations of
122 mixed ancestry) might have lower mortality rates of ALS. In a population-based study in Cuba, ALS
123 mortality rate was 0.55 per 100,000 individuals in a mixed population, but was about 0.9 per 100,000
124 individuals in white or black individuals ¹⁶, confirming the importance of ancestral origin in disease risk.

125 In Europe, most men have spinal onset disease, and women have increased propensity for bulbar onset
126 disease ⁹. The percentage of individuals with bulbar onset disease is much lower in Asia compared with
127 Europe, but a North to South gradient has been described in Europe, with higher percentage of
128 individuals with spinal onset disease in Southern Europe ⁹. Based on available data, the age of diagnosis
129 and first symptoms is higher in Europe compared to Asia and South America. In Europe, the age of
130 onset peaks at 65 ⁹. The main limitation of global ALS epidemiology is that almost 80% of studies have
131 been conducted in Europe and the US, and mainly comprise patient cohorts of Northern European
132 ancestry. International consortia collecting data in areas with mixed populations and in different
133 continents will be required to fully elucidate the range of clinical presentations, and to understand the
134 roles of ancestry, genetics and environmental exposures in ALS causation.

135

136 **[H2] Causes of ALS**

137

138 **[H3] Genetics.** ALS is considered a complex genetic disorder with a Mendelian pattern of inheritance in a
139 proportion of cases, but no discernible family history in the rest. Mathematical models developed using
140 population-based registers have suggested that individuals with ALS are likely to carry a number of ‘at
141 risk’ variants that interact with environmental factors through a series of at least 6 notional steps
142 leading to disease manifestation. One of these steps is thought to be the genetic risk (from birth), but
143 the interplay of environmental factors that lead to the remaining steps have yet to be defined. In
144 transgenic mice, the genetic background can alter the phenotypic presentation of ALS ^{17,18}, suggesting
145 that human disease phenotypes could also have a genetic basis, and that genomic and epigenomic
146 “fingerprinting” could permit the clustering of different phenotypic manifestations into discrete
147 underlying causes that are amenable to therapeutic intervention.

148

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

157
158 Of the known genes of major effect for the development of ALS (Table 1 [OK), our current knowledge
159 comes primarily from the study of ancestral European (Europe, USA, Canada and Australia) and East
160 Asian populations; within these populations, the dichotomization of ALS into ‘familial’ and ‘sporadic’
161 subtypes is an over-simplification. Although at least 30 genes are known to confer a major risk for ALS,
162 evidence suggests a role of oligogenic inheritance (in which a phenotypic trait is determined by more
163 than one gene) and of genetic pleiotropy (in which a single gene has multiple phenotypic
164 manifestations). Within populations of European extraction, up to 20% of people with ALS have a family
165 history of either ALS or FTD (Familial ALS) , and of these 4 genes account for up to 70% of all cases of
166 familial ALS , namely *C9orf72*, *TARDBP* (also known as *TDP43*), *SOD1* and *FUS* ²⁰. However, even in the
167 case of these known Mendelian inherited genes, familial forms of ALS are often characterized by lower
168 than 50% penetrance [and genetic pleiotropy, with evidence of oligogenic and polygenic inheritance in
169 individuals with apparently sporadic disease ^{21, 22}.

170
171 **[H3] Environmental and lifestyle factors.** Epidemiological case control studies have sought to
172 determine the environmental causes of ALS. Early epidemiological studies from regions with a high
173 incidence of ALS and dementia such as Guam and the Kii peninsula of Japan suggested a role for
174 neurotoxins contained within cycad seeds, including β -methylamino-L-alanine . Although the role of β -
175 methylamino-L-alanine²³ has not been substantiated, a possible role for related cyanotoxins has been
176 proposed, and exposure to water harbouring cyanobacterial blooms has been suggested to contribute
177 to risk of ALS in susceptible individuals ²⁴.

178
179 ALS has been reported at a higher frequency among groups of athletes compared to the general
180 population although whether physical activity is a risk factor for ALS, or a marker of underlying athletic
181 prowess is unclear. Evidence from a UK study suggests that individuals with ALS had higher rates of pre-
182 morbid physical activity, but two other European studies suggested either no effect, or a protective
183 effect ²²⁻²⁴. Reasons for this discrepancy might relate to study design and true population-based
184 differences. However, because ALS is a rare disease, smaller case control studies are often
185 underpowered and are subject to both bias and error in interpretation. To address these problems in
186 study design, a very large case control study has been completed as part of the EuroMOTOR project
187 (www.euromotorproject.eu), which has collected >1,500 population-based incident cases and 3,000
188 matched controls across 3 countries. Analysis is ongoing, although preliminary data suggest that

189 exposure to smoking might increase the risk of developing ALS, but type 2 diabetes mellitus, high levels
190 of circulating lipids and exposure to female contraceptive hormones seem to be protective ^{25, 26}

191 **[H1] Mechanisms/pathophysiology**

192

193 **[H2] Histopathology**

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

200 In most subtypes of ALS the tar DNA-binding protein 43 (TDP-43) is the major constituent of these
201 inclusions, although mutations in *TARDBP* are a rare cause of ALS ^{27, 28} Indeed, approximately 97% of
202 patients with ALS have features of a TDP-43 proteinopathy, with depletion of TDP-43 in the nucleus, but
203 the formation of cytoplasmic aggregates with skein-like or compact morphology in residual motor
204 neurons (Figure 2A). In specific subtypes of ALS, other types of protein aggregates might be seen, such
205 as P62-positive, TDP-43 negative protein inclusions that are caused by dipeptide repeat proteins and
206 might be seen outside the motor system in patients with (Figure 2C) and neurofilamentous hyaline
207 conglomerate inclusions (Figure 2B) and the accumulation of misfolded superoxide dismutase (SOD1) in
208 patients with SOD1-ALS Although protein aggregates are the hallmark of ALS, the high molecular weight
209 **YES]** complexes that precede the formation of the aggregates, rather than the aggregates themselves²⁹,
210 ³⁰, might be the toxic species. Shedding of higher molecular protein complexes might mediate cell to cell
211 propagation of disease, linking the progression of ALS to a prion-like mechanism, as has also been
212 suggested for tau and synuclein-mediated diseases ^{31, 32}.

213

214 The gross pathological features of ALS comprise skeletal muscle atrophy, atrophy of the motor cortex
215 and pallor and sclerosis of the corticospinal and corticobulbar tracts), together with thinning of the
216 hypoglossal nerves (which are involved in the control of the muscles of the tongue) and the ventral roots
217 of the spinal cord. Microscopic examination usually reveals a depletion of at least 50% of spinal motor
218 neurons and diffuse astrocytic gliosis and microglial infiltration in the grey and white matter of the
219 spinal cord (Figure 2D AND 2F). Axonal loss, gliosis and myelin pallor are seen in the corticospinal tracts,

220 and astrocytic gliosis is usually observed in the motor cortex, together with variable depletion of upper
221 motor neurons. Skeletal muscle shows features of denervation and reinnervation, with fibre type
222 grouping and clusters of angular atrophic fibres.

223

224

225 **[H2] Overview of pathophysiology OK**

226 Progress has been made in the identification of the genetic causes of ALS^{21, 22} and models in rat, mouse,
227 zebrafish, flies, worms and yeast have been developed to study the mechanisms by which gene
228 mutations cause motor neuron degeneration and to model particular biological processes thought to be
229 important in disease pathobiology. All of these models have limitations and none fully recapitulates
230 human disease, which is partly because most models are based on gene overexpression (with multiple
231 copies of the human variant inserted into the transgenic model) and because the human neuro-axis
232 differs substantially from that of lower animals. Nevertheless, findings from animal models can
233 contribute to our understanding of the cell biology underlying neurodegeneration and can open new
234 avenues towards targeted drug development. In reality, the cellular disruption in ALS is likely the result
235 of many different interacting mechanisms that culminate in larger network disruption, and the
236 separation of different mechanisms is somewhat artificial. This is exemplified by the finding that
237 multiple factors can contribute to neuronal damage in models of *Sod1* **OK MODIFIED BY PJS ?]**
238 mutations (Table 1). The relative extent by which each of these factors contributes to the overall
239 pathobiology of human disease cannot be fully ascertained, it would be erroneous to assume that all of
240 these factors are involved in all cases of ALS, as human disease is heterogeneous. Notwithstanding, each
241 of the thematic areas should be considered in detail, as they represent our current knowledge base of
242 the pathophysiology of ALS, and are the drivers of current and future therapeutic initiatives (Figure 3).

243

244 **[H2] Impaired protein homeostasis**

245 Mutations in some genes lead to the translation of proteins that are misfolded, have an abnormal
246 cellular localization or are aberrantly formed, and that can directly or indirectly impair the proteasome
247 or autophagy machinery of the cell, leading to impaired cellular protein turnover. Indeed, genes
248 associated with familial ALS encode proteins that can promote dysfunction of the ubiquitin-proteasome
249 system. For example, mutant SOD1 is associated with reduced expression of ubiquitin-proteasome
250 system components³³, valosin-containing protein (VCP) and ubiquilin-2 are involved in substrate
251 delivery to the proteasome, and this function is disrupted in the presence of ALS-associated mutations

252 ³⁴⁻³⁶. In addition, dysregulation of chaperone proteins has been identified in ALS associated with *SOD1*
253 and *TARDBP* mutations ³⁷⁻⁴⁰ . Mutations in *VAPB* (encoding vesicle-associated membrane protein
254 associated protein B) can cause defective activation of the unfolded protein response in disease models
255 ^{41, 42} .
256
257 *C9orf72* is a key regulator of autophagy initiation ⁴³ and loss of this function might contribute to the
258 presence of ubiquitin and p62 positive, TDP-43 negative inclusions in extra-motor areas of the central
259 nervous system (CNS) in *C9orf72*-related ALS. Sequestosome-1, optineurin and ubiquilin-2 have a role in
260 the early steps of autophagy ⁴⁴⁻⁴⁶ , and alsin, polyphosphoinositide phosphatase (FIG4), transitional
261 endoplasmic reticulum ATPase (VCP) and charged multivesicular body protein 2b (CHMP2B) have roles
262 in the maturation of autophagosomes into autophagolysosomes by regulating the fusion of
263 autophagosomes with multivesicular bodies, endosomes and lysosomes ⁴⁷⁻⁵¹ . Mutations in
264 *SQSRM* might disrupt the correct delivery of autophagic substrates to the autophagosome ⁵² and
265 mutations in *UBQLN2* and *OPTN* (which both encode autophagy receptors) are also associated with ALS.
266 The activities of sequestosome-1 and optineurin are regulated by serine/threonine-protein kinase
267 (TBK1) and ^{53, 54} haploinsufficiency of *TBK1* [YES is a cause of familial ALS, which supports the hypothesis
268 that reduced substrate delivery to autophagosomes might contribute to motor neuron injury in ALS.
269 Reduced VCP activity has been shown to decrease the maturation of autophagosomes. Other proteins
270 implicated in ALS pathophysiology, including alsin and FIG4 , can affect autophagy at the stage of
271 initiation, although the mechanism for this is unclear^{47, 55} . Both *SOD1* and TDP-43 are known substrates
272 of autophagy, suggesting that defective autophagy could contribute to the toxic accumulation of these
273 proteins in ALS. The formation of dipeptide repeat proteins through repeat-associated non-ATG (RAN)
274 translation from the expanded RNA repeat of the *C9orf72* gene might also result in dysproteostasis, but
275 this remains to be conclusively demonstrated and the mechanism elucidated.

276
277 [OK

278 279 [H2] Aberrant RNA metabolism

280 Alteration of mRNA processing is a key theme in ALS pathogenesis⁵⁶ . mRNA undergoes a complex system
281 of processing as it transits from the nucleus to cytoplasm, where it is translated into protein. In neurons,
282 mRNAs can be transported to allow local translation in the axonal compartment. Although the
283 functional consequences of RNA dysregulation that lead to age-related and selective degeneration of

284 neuronal populations remain poorly understood, analysis of the transcriptome of actively transcribing
285 mRNAs will be essential in elucidating the upstream molecular events contributing to neuronal injury.

286

287 The discovery of mutations in *TARDBP* and *FUS* as rare causes of ALS has identified a crucial
288 pathogenetic role for RNA binding proteins that contain low complexity domains⁵⁷. Mutant TDP-43 or
289 FUS proteins mislocalize from the nuclear to the cytoplasmic compartment and this is hypothesised to
290 result in the loss of the normal processing of their target RNAs^{58,59}. Indeed, up to one third of the
291 transcriptome is altered in models of TARDBP-related ALS⁶⁰, and dysregulation of gene expression has
292 also been observed in relation to mutations in *C9orf72*, *SOD1*, and *FUS*⁶¹, including transcription,
293 alternative splicing of mRNA, axonal transport of mRNAs and biogenesis of microRNAs^{62,63}.

294

295 The GGGGCC repeat expansion in the noncoding region of *C9orf72* forms stable parallel uni- and
296 multimeric G-quadruplex structures, which avidly interact with RNA processing factors^{64,65}. In addition,
297 the repeat expansion gives rise to abnormal RNA species that can be identified as nuclear RNA foci and
298 the *C9orf72* mutation might induce direct RNA toxicity, by, for example, sequestering RNA binding
299 proteins⁶⁶⁻⁶⁸. Indeed, a large set of proteins that bind to the expanded repeat have been identified⁶⁹.
300 In addition, repeat expansions could lead to the formation of R-loops (that is, DNA-RNA hybrid
301 structures) that increase susceptibility to DNA damage and genome instability^{70,71}. Indeed, R-Loops and
302 genome instability due to double strand DNA breaks and defective serine-protein kinase ATM-mediated
303 DNA repair have been identified as important components of neuronal injury due to GGGGCC repeat
304 expansion in *C9orf72*⁷².

305

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

314

315 Mutations in other genes involved in RNA metabolism: such as *TAF15*, *EWSR1*, *hnRNPA1*, *hnRNPA2B1*
316 and *MATR3* have been implicated in ALS ^{82, 83}. The mislocalization of the mutant proteins into the
317 cytoplasm might result in a toxic gain-of-function, and the effect of these proteins on the formation of
318 stress granules is an area of intense research effort ⁸⁴⁻⁸⁶ .

319

320 [H2] Nucleocytoplasmic and endosomal transport

321 In addition to altering RNA metabolism , the GGGGCC repeat expansion in *C9orf72* is believed to alter
322 the intracellular localisation of *C9orf72* mRNA. Dipeptide repeat proteins are generated from the repeat
323 expansion in *C9orf72* and interfere with proper nucleocytoplasmic transport and trigger neurotoxicity
324 via several mechanisms ^{87, 88}. For example, arginine-rich dipeptide repeat proteins isolated from *C9orf72*
325 expansions can induce phase separation of proteins that have a role in RNA and stress granule
326 metabolism, and produce spontaneous stress granule assembly ⁸⁹. In addition, increased binding of
327 mRNA export adaptors to expanded *C9orf72* pre-mRNAs might target those pre-mRNAs for nuclear
328 export, which could allow RNA translation to occur with potential toxicity from the expression of
329 abnormal dipeptide repeat protein species ^{68, 90}. Indeed, sequestration of the nuclear export adaptor
330 serine/arginine-rich splicing factor 1 (SRSF1) by the repeat expansion region of the RNA, triggers nuclear
331 RNA export factor 1 (NXF1)-dependent nuclear export of *C9orf72* transcripts retaining the
332 hexanucleotide repeats, allowing RAN translation to dipeptide repeats in the cytoplasm . Depletion of
333 SRSF1 in cellular and *in vivo* models reduces the production of dipeptide repeat proteins and
334 neurotoxicity ⁹¹.

335

336 [H2] Endosomal and vesicle transport

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

343

344 [H2] Axon structure and function

345 The finding of *DCTN* (encoding dynactin) , *PFN1* (encoding profilin 1) and *TUBA4A* (encoding tubulin
346 alpha-4A chain) mutations suggests that abnormalities of proteins that are essential for axonal

347 transport are associated with ALS ⁹⁵⁻⁹⁷. In addition, mutations in *NEFH* (encoding neurofilament) have
348 also been described in a small number of patients ⁹⁸, although whether these mutations are
349 pathogenetic through axonal dysfunction remains to be seen. Rare mutations in *PRPH* encoding
350 peripherin, another cytoskeletal protein, have been suggested to have a role in ALS pathogenesis,
351 possibly through effects on neurofilament housekeeping including protein cargo trafficking ^{99, 100}.

352

353 **[H2] DNA repair**

354 Impaired DNA repair was suggested to have a role in ALS pathophysiology following the identification of
355 *FUS* mutations, although the exact role of DNA repair failure in ALS remains to be clarified^{101, 102}.
356 Mutations in *NEK1* and *C21orf2* , both of which encode proteins involved in DNA repair, have recently
357 been identified as causes for ALS ¹⁰³⁻¹⁰⁵ although the biological pathways associated with their their
358 causal role awaits confirmation.

359

360 **[H2] Excitotoxicity**

361 Motor neurons are very sensitive to toxicity induced by calcium entry following excessive glutamate
362 stimulation as they have a lower calcium buffering capacity than other neuronal subtypes and α -amino-
363 3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that are more calcium permeable (as
364 they contain less of the GluR2 subunit) ¹⁰⁶. In addition, excitatory amino acid transporter 2 (EAAT2), an
365 astroglial [protein that is the main synaptic glutamate re-uptake transporter, is impaired in ALS, which is
366 likely to result in synaptic glutamate abundance and motor neuron toxicity. The loss of EAAT2 has been
367 observed in both rodent models and patients with familial or sporadic ALS. Excitotoxicity is thought to
368 be a mechanism common to all forms of ALS, although the evidence for this remains indirect. One
369 argument is that riluzole, which can attenuate disease progression and is an approved drug for
370 neuroprotection in ALS, can inhibit glutamate release ^{107, 108}. However, whether this underlies the
371 therapeutic effect of riluzole remains unclear.

372

373 **[H2] Oligodendrocyte degeneration**

374 Oligodendrocyte degeneration has been observed in ALS. In the healthy CNS, oligodendrocytes are
375 replaced by the proliferation of oligodendrocyte precursor cells, which are abundantly present ^{109, 110} . At
376 least in animal models of ALS, and for reasons that are now clear, oligodendrocyte precursor cells fail to
377 go through the final stages of differentiation. Oligodendrocytes provide vital metabolic support to axons
378 through the shuttling of lactate through monocarboxylate transporter 2 ^{111, 112}, and accordingly,

379 dysfunction of oligodendrocytes contributes to the motor axonal failure in ALS. Restoring
380 oligodendrocytic function by transgenically deleting mutant SOD1 from these cells significantly slows
381 disease progression and prolongs their life span ¹¹³. In patients with ALS, abnormalities in
382 oligodendrocytes can occur, but whether these changes contribute to the disease remains to be
383 demonstrated.

384

385 **[H2] Neuroinflammation**

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

397 **[H2] Mitochondrial dysfunction**

398 Mitochondrial function is impaired in ALS and changes in mitochondrial morphology have been shown in
399 some patients, and in the SOD1 mouse model ^{118,119}. In the SOD1 model, vacuoles containing protein
400 aggregates containing mutant SOD1 can be observed in the mitochondrial inter-membrane space,
401 leading to impairment of protein import ¹²⁰. In addition, oxidative damage to mitochondrial proteins
402 leads to defects in respiratory chain function in patients with ALS and in SOD1 mouse models ¹²¹, and
403 various experimental models of ALS have defects in axonal transport of mitochondria, which could
404 contribute to the axonopathy at the neuromuscular junction ^{122,123}.

405

406 Many of the functions disrupted in ALS are regulated by signalling between the endoplasmic reticulum
407 and mitochondria, underpinned by tight junction associations mediated by the endoplasmic reticulum
408 protein VAPB and the outer mitochondrial protein regulator of microtubule dynamics protein ¹²⁴. These
409 associations are perturbed by *TARDBP* and *FUS* mutations ^{125,126}. TDP-43 preferentially binds to mRNAs
410 encoding respiratory chain complex 1 subunits and causes complex 1 disassembly ¹²⁷ and accumulates in

411 the mitochondria of patients with ALS and mutations in *TARDBP* increase the mitochondrial localization
412 of TDP-43. Suppression of TDP-43 localization to mitochondria improves mitochondrial dysfunction and
413 reduces neuronal loss in mTDP-43 cell based models. In C9orf72-related ALS models, the dipeptide
414 repeat protein poly(GR) appears to compromise mitochondrial function and causes oxidative stress and
415 DNA damage ¹²⁸. *CHCHD10* mutations, which are associated with familial ALS, can promote the loss of
416 mitochondrial cristae junctions, impair mitochondrial genome maintenance and interfere with apoptosis
417 by preventing of cytochrome-C release ¹²⁹.

418

419 **[H2] Final common pathway**

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

426

427 Whatever the mechanisms of ALS, the end result is that the motor neuron cannot maintain its axonal
428 projections, leading to axonal retraction and denervation of the target cell. For lower motor neurons,
429 this results in denervation of the muscle, but for upper motor neurons results in the loss of proper
430 control of lower motor neurons, hypertonicity and weakness .. In addition, a loss of important neural
431 networks within motor and extra-motor domains is also apparent ¹³⁰. As many of the proteins encoded
432 by genes that are implicated in ALS are ubiquitously expressed (Table 1), it is unclear why motor neurons
433 are the most susceptible to the hazardous effects of these mutations. The large size of motor neurons,
434 and in particular the need to maintain their long axonal projections, could make these cells more
435 sensitive to metabolic abnormalities than others, but other neuronal subtypes, such as sensory neurons,
436 have even larger axonal projections. Other factors that have been suggested to have a role are the high
437 expression of EphA4 and matrix metalloprotein 9 and the low expression of osteopontin and insulin-like
438 growth factor 2 by motor neurons, which might limit axonal sprouting and repair. Of particular interest
439 is that within the motor neuron pool, neurons that establish the fast fatiguable motor units die first in
440 ALS ^{131, 132}, but how this relates to the other vulnerability factors needs to be clarified.

441

442 **[H1] Diagnosis, screening and prevention**

443

444 **[H2] Clinical presentations**

445 The clinical hallmark of ALS is the involvement of both upper and lower motor neurons (Figure 1).
446 Patients can present with symptoms of an upper motor neuron predominant onset (that is, spasticity
447 and weakness) in whom lower motor neuron involvement only becomes evident at later stages of
448 disease.^{7, 133-136} . Conversely, patients can present with symptoms of lower motor neuron dysfunction,
449 which includes fasciculations, cramps and muscle wasting. Approximately one third of patients with ALS
450 present with bulbar-onset disease, which is characterized by progressive dysarthria, followed by
451 difficulty swallowing and often with associated emotional lability. Limb onset disease accounts for 60%
452 of cases, is usually asymmetrical in presentation and can first develop in the upper or lower limb. [Up to
453 5% of patients present with respiratory problems and are often seen first in cardiology and pulmonology
454 clinics prior to their referral to neurology clinics¹³⁷. In these cases, patients can also present with
455 unexplained weight loss. Evidence suggests that some patients with ALS are hypermetabolic;¹³⁸
456 although the pathophysiology underpinning this is not well understood. Cardiovascular risk factors
457 (such as hyperlipidemia or obesity) might attenuate risk¹³⁸, but do not alter clinical outcome¹³⁹ .
458 Patients can present with a pure motor phenotype of ALS, and have normal cognition and behaviour,
459 but some patients can present with a purely cognitive or behavioural phenotype consistent with
460 frontotemporal dementia(FTD)), or a mixed phenotype with minor changes in executive impairment
461 that progress over time. Frontotemporal dementia is part of the presenting features of 13% of incident
462 cases²⁻⁴ and approximately 30% of all incident patients have some evidence of executive dysfunction at
463 the time of first presentation^{3, 140}. Depending on the population and the extent of cognitive testing
464 performed, most studies have suggested that up to 50% of patients can remain cognitively normal
465 throughout the course of the disease³ Behavioural changes are common in patients with ALS, with
466 apathy as the most prevalent symptom. Detailed examination of behavioural changes in patients with
467 ALS, using a disease specific behavioural scale (that is, the Beaumont Behavioural Index) suggests that
468 up to 40% of incident cases have new behavioural changes that can be clustered into at least 5 different
469 groups which roughly map to known neuroanatomical networks and pathways¹⁴¹. Substantial
470 autonomic impairment (such as cardiovascular, gastrointestinal and bladder dysfunction) does not occur
471 in the majority of patients with ALS.

472

473 **[H2] Diagnostic criteria**

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

479
480 Diagnosing ALS is based on the El Escorial criteria (Box 2) ¹⁴². Diagnosis according to these criteria
481 requires a history of progressive weakness spreading within a region or to other regions (such as bulbar
482 regions (speech and swallowing), cervical regions (upper limbs), thoracic regions (chest wall and
483 abdominal muscles) or lumbar regions (lower limbs), with evidence of lower motor neuron (through the
484 presence of specific symptoms or evidence of denervation on electromyography) and upper motor
485 neuron (through the presence of specific symptoms and brisk deep tendon reflexes) involvement. In the
486 original criteria, diagnostic certainty ranged from Suspected ALS, (although this is no longer included in
487 the revised criteria), to Definite ALS (in which three body regions with mixed upper and lower motor
488 neuron findings were observed), which relates to the burden of disease. Neurophysiological findings
489 have been classified using the Awaji Criteria, which can enhance diagnostic and prognostic sensitivity ¹⁴³.
490 Variants of the El Escorial criteria are used in research settings and for the purposes of clinical trial
491 enrolment, but these criteria should not be routinely used in clinical practice for routine patient
492 management, as “possible ALS” described by the criteria is almost always ALS clinically ^{144, 145}. Genetic
493 testing can also be included in patients with a strong family history of ALS ¹⁴⁶ and clinical evidence of
494 disease, although this is not uniformly applied across centres ¹⁴⁷.

495
496 **[H2] Cognitive and behavioural deficits**
497 Standard diagnostic and stratification parameters for ALS do not yet include cognitive or behavioural
498 status, which is altered in up to 50% of cases (depending on the extent of cognitive and behavioural
499 assessment ²⁻⁴). Various screening tools have been designed to identify patients with ALS and cognitive
500 and behavioural changes in the clinic, such as the Edinburgh Cognitive and Behavioural ALS Screen
501 (ECAS), which is validated in several languages and is widely used, as it has a high degree of sensitivity
502 with lower degrees of specificity ¹⁴⁸. Individuals with abnormal ECAS scores (after adjustment to
503 population-based and educational norms) should be referred for a full neuropsychological evaluation ¹⁴⁹.
504 The detection of cognitive and behavioural changes is important for patients with ALS and their

505 caregivers, as executive impairment is associated with a more-rapid disease trajectory and behavioural
506 changes are associated with higher caregiver burden¹⁵⁰.

507

508 **[H2] Biomarkers**

509 As ALS is a clinical syndrome with a heterogeneous phenotypic manifestation [and clinical course,
510 diagnostic and prognostic biomarkers are urgently required for the purposes of stratification. Levels of
511 neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain in the cerebrospinal fluid
512 (CSF) can differentiate patients with ALS from those with mimics including cervical myelopathy,
513 multifocal motor neuropathy and inclusion body myositis, with moderate sensitivity and specificity, and
514 levels have a moderate correlation with disease progression¹⁵¹⁻¹⁵³. However, CSF neurofilament levels
515 are not integrated into standard clinical practice. Levels of NfL in serum are sensitive and specific for
516 separating patients with ALS from healthy controls, but data on comparison with ALS mimics are not
517 available.

518

519 MRI studies of patients with ALS have shown corticospinal tract degeneration, with extensive
520 involvement within the frontal and temporal regions and basal ganglia, compared with controls
521 Evidence suggests that selective network vulnerability of structural and functional ‘connectomes’ could
522 drive the clinical manifestations of ALS, such as vulnerability of the corticospinal, orbitofrontal,
523 orbitotemporal and frontostriatal circuits¹⁵⁴⁻¹⁵⁶. The presence of network disruption is also supported by
524 findings using spectral electroencephalogram¹³⁰, and that patients with different degrees of cognitive
525 impairment show significantly different patterns of frontal lobe metabolic impairment on ¹⁸F
526 fluorodeoxyglucose PET imaging¹⁵⁷. However, neither imaging nor spectral electroencephalogram can
527 provide individualised data that can be used as a reliable biomarker of upper motor neuron dysfunction
528 and of cognitive impairment in patients with ALS.

529

530 **[H2] Differential diagnosis**

531 The differential diagnosis in patients with pure bulbar pure upper motor neuron or pure lower motor
532 neuron presentations includes ALS variants, treatable ALS mimics and disorders with a more benign
533 prognosis^{134, 158}. Other forms of motor neuron disease include progressive muscular atrophy (that is, the
534 exclusive degeneration of lower motor neurons) and primary lateral sclerosis (that is, the exclusive
535 degeneration of upper motor neurons). Some patients with progressive muscular atrophy have
536 mutations in genes associated with ALS¹⁵⁹. Similarly, patients with primary lateral sclerosis may have a

537 family member with ALS and most autopsies of patients with primary lateral sclerosis show subtle signs
538 of ALS pathology in the lower motor neurons within the brain stem and spinal cord^{135, 158}.

539
540 Several conditions have similar initial clinical features as ALS and should be considered in the differential
541 diagnosis¹⁴⁵, including cervical myelopathy, multifocal motor neuropathy, myasthenia gravis, Lambert
542 Eaton myasthenic syndrome and inclusion body myositis. Features that should alert the clinician to a
543 possible mimic syndrome include presentation with of symmetrical findings; prominent extensor plantar
544 responses (which should raise suspicion of a cervical myelopathy) and the presence of sensory findings.
545 Although sensory symptoms are common in ALS, clinical evidence of sensory loss is atypical and should
546 trigger further investigations. In addition, the presence of substantial weakness in the absence of
547 wasting – which is common in multifocal motor neuropathy and myasthenia gravis – and the presence
548 of disproportionate involvement of quadriceps – which is common in inclusion body myositis – may
549 indicate the presence of an ALS mimic syndrome¹⁶⁰. As ALS is a progressive disease, failure of the
550 condition to progress over months should also trigger a re-investigation¹⁶¹.

551

552 **[H2] Staging and prognosis**

553 Several different staging systems for ALS have been described (Figure 4)¹⁶²⁻¹⁶⁵, including the King's
554 system, which is based on the number of affected regions of the body, and the Milano-Torino system
555 (MITOS), which is based on a clinical scale. The prognosis of ALS is highly variable and prognostic
556 algorithms have been generated from population-based and clinical trial-based datasets^{166, 167}. Negative
557 prognostic indicators include bulbar or respiratory onset disease, the presence of executive impairment
558 or frontotemporal dementia and weight loss. Several biochemical markers of prognosis have been
559 reported including serum urate, serum creatinine, serum chloride, and increased serum and CSF
560 neurofilament levels^{153, 168-170}. Declining respiratory function, measured by slow vital capacity, forced
561 vital capacity and sniff nasal inspiratory pressure also correlate with short survival^{166, 167, 171, 172}.

562 **[H2] Clinical genetics and predictive testing**

563 Consensus guidelines recommend genetic testing of probands with ALS who have a first or second
564 degree relative with ALS and/or frontotemporal dementia^{19, 173}. As the genetic risk for ALS depends on
565 ancestral origin, the genetic testing should be contextualized; for example, *C9orf72* variants are rare in
566 Asia, whereas mutations in *OPTN* are more common in Asian than in European populations. Although
567 the potential benefits of genetic testing for patients are clear and could improve knowledge about their
568 disease, family planning and their possible inclusion in clinical trials, individuals also have a right not to

569 know their genetic status. Pre-symptomatic testing of family members of patients with ALS remains
570 controversial. Guidelines for genetic testing in research settings have been published ¹⁷⁴, but most
571 centres do not advocate routine testing outside of specialist centres ¹⁴⁷.

572 **[H1] Management**

573
574 ALS management is best achieved by a multidisciplinary approach to care, comprising a clinical team
575 with different specialities, including neurologists, psychologists, nutritionists, pulmonologists, physical
576 therapists, speech therapists and specialized nurses^{175, 176}. Multidisciplinary care increases survival ¹⁷⁷⁻¹⁷⁹,
577 reduces the number of hospital admissions and shortens hospital stays ¹⁷⁸ and increases quality of life of
578 patients with ALS ¹⁸⁰. This is likely related to the optimization of pharmacological and non-
579 pharmacological interventions and enhanced adherence to treatment guidelines.

580

581 **[H2] Disease-modifying therapies**

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

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

598

599 [H2] Symptomatic treatments

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

604
605 [H3] **Spasticity.** Spasticity is present in most patients with ALS, but only a small proportion need
606 treatment. The most commonly used drugs are baclofen and tizanidine (both of which are muscle
607 relaxants) although no randomized controlled trials in patients with ALS have been conducted. When
608 patients have severe, disabling spasticity, baclofen can be administered through an intrathecal pump. .
609 Cannabinoids have been approved for the treatment of spasticity in patients with multiple sclerosis and
610 are also used off-label or as a self-prescribed medication in patients with ALS¹⁸⁷.

611
612 [H3] **Sialorrhoea.** Sialorrhoea (that is hypersalivation), causing drooling and the pooling of saliva within
613 the oral cavity is one of the most disturbing symptoms in patients with ALS, and is more commonly
614 observed in patients with bulbar-onset disease and during late-stages. Sialorrhoea can be treated [with
615 anticholinergic drugs, such as scopolamine, atropine, hyoscine, amitriptyline and glycopyrrolate.
616 Adverse effects associated with the use of anti-cholinergics include blurred vision, mouth dryness and
617 constipation, and these drugs are contraindicated in patients with heart conduction disturbances and
618 prostatic hypertrophy. In patients in whom pharmacological treatments are ineffective or are not
619 indicated, botulinum toxin A or B injections into the salivary glands can be used to treat sialorrhoea^{188, 189}.
620 Salivary gland irradiation has been also proposed ¹⁹⁰.

621
622 [H3] **Pain.** Pain is reported in 15–85% of patients with ALS, depending on the duration of the disease
623 and the setting of the study, and is more frequently of nociceptive than of neuropathic origin. ¹⁹¹
624 Depending on the type of pain, pharmacological treatments include gabapentin, pregabalin and tricyclic
625 antidepressants (for neuropathic pain), and NSAIDs, opioids and cannabis for nociceptive pain), but no
626 randomized controlled trials evaluating treatment of pain in patients with ALS are available. Nociceptive
627 pain can be also treated with intra-joint injections of lidocaine or steroids, and physical therapy,
628 including assistive range-of-motion exercises.

629

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

637

638 **[H3] Dysphagia**

639 Dysphagia is reported by about 60% of patients with spinal onset ALS, within two years from onset and
640 100 % of patients with bulbar-onset disease ¹⁹⁴. Several strategies can be implemented to reduce the
641 effects of dysphagia in patients, including dietary changes such as modification of the consistency of the
642 diet, the use of fluid thickeners and prescription of high-protein and high-caloric supplements,
643 swallowing facilitating manoeuvres and exercises (such as oral and pharyngeal range-of-motion
644 exercises, head postures and the technique of supraglottic swallow). An option for severe difficulties
645 with swallowing is to use enteral nutrition via the insertion of a gastrostomy tube. No established
646 criteria are available for the initiation of enteral nutrition in patients with ALS, but weight loss of >5% or
647 unsafe swallowing are generally considered to be red flags that should prompt intervention. ¹⁷⁵. Several
648 techniques are available for minimally invasive tube insertion and open surgery is not recommended ¹⁹⁵,
649 ¹⁹⁶. Parenteral nutrition provided through a central venous catheter is an alternative to enteral nutrition
650 in patients with ALS who have severe respiratory insufficiency for whom PEG are contraindicated ^{197, 198}.

651

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

660

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

666
667 **[H3] Mood alterations.** Depression is a relatively common symptom in patients with ALS and has been
668 found in up to 50% of patients. Depression is generally treated with selective serotonin reuptake
669 inhibitors (SSRI) or tricyclic antidepressants. Pseudobulbar affect (that is, episodes of uncontrollable
670 crying or laughing) is a distressing symptom that has been reported in up to 50% of patients with ALS²⁰⁴
671 [and can be treated with SSRIs and tricyclic antidepressants, although this is off-label.
672 Dextromethorphan (a sigma-1-receptor agonist and an uncompetitive NMDA receptor antagonist) and
673 low-dose quinidine were effective in reducing symptoms of pseudobulbar affect by 50% in patients with
674 ALS or those with multiple sclerosis²⁰⁵.

675
676 **[H3] Cognitive impairment.** Cognitive impairment, in particular frontotemporal dementia, is one of the
677 most disabling symptoms in patients with ALS. No pharmacological therapy is effective for the treatment
678 of frontotemporal dementia, and acetylcholinesterase inhibitors, which are used for Alzheimer disease,
679 are not effective. However, some symptoms of frontotemporal dementia can be pharmacologically
680 treated; evidence suggests SSRIs might help to control the loss of inhibition, overeating and compulsive
681 behaviour, and antipsychotics can be used to reduce restlessness. Education of caregivers about the
682 symptoms of frontotemporal dementia can be useful to help the management of patients at home²⁰⁶.

683
684 **[H3] Respiratory insufficiency.**
685 The vast majority of patients with ALS die from respiratory failure. Non-invasive ventilation is the
686 symptomatic treatment of choice for respiratory failure, and provides significantly longer survival
687 compared to those who do not use NIV (316 vs 229 days) and improves quality of life^{207 208}. Accepted
688 criteria for starting non-invasive ventilation are symptoms or signs related to respiratory muscle
689 weakness (such as, dyspnoea, orthopnoea or daytime fatigue), a vital capacity of < 80% of predicted
690 levels, PaCO₂ > 45 mmHg, SaO₂ < 90% during ≥ 5% of sleep time¹⁷⁶. One distressing symptom that is
691 related to respiratory muscle weakness in patients with ALS is the inability to cough effectively. This can

692 be controlled by the use of cough-assist devices, such as the breath-stacking technique or a mechanical
693 insufflator-exsufflator ²⁰⁹.

694

695 **[H2] End of Life Management**

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

713

714 **[H1] Quality of Life**

715

716 Much of the effort of physicians and other health care providers is focused on optimizing the quality of
717 life (QOL) of patients with ALS. The choice of a specific QOL instrument is complex, and has been
718 reviewed ²¹⁰. The perception by individuals with ALS of their QOL takes shape at the time of disclosing
719 the diagnosis, and can be influenced by the manner in which they are informed . Well-recognized
720 systematic approaches are available, such as the SPIKES approach, that can convey the diagnosis in a
721 less distressing manner and can leave the patient feeling hopeful and supported ²¹¹⁻²¹³.

722

723 Health-related QOL (HRQOL) refers to an individual's perception of their QOL as a function of physical
724 and mental well-being²¹⁴; measures of HRQOL generally decline as ALS advances^{210, 215}. In contrast,
725 OQOL encompasses medical factors and a wide variety of non-medical factors, such as family, friends,
726 occupation, financial well-being, spirituality or religion and existential concerns²¹⁶. Patients with ALS
727 often view their OQOL as good, which persists despite the progression of physical disability^{217, 218}. This
728 might be explained by a 'response shift' (also called a frame shift or well-being paradox), whereby the
729 individual recalibrates the factors that are deemed meaningful to maintenance of their QOL. Most
730 commonly, this centres around the decreased importance of physical activities and the greater role of
731 interactive and existential factors, such as social relationships and spirituality²¹⁹⁻²²¹. However, not all
732 patients maintain a high QOL with advancing illness. Many factors can negatively affect QOL in patients
733 with ALS, identifying potential areas for intervention, although other factors can improve QOL (Figure 5)
734 180, 207, 214, 222-228

735
736 Despite good QOL of patients with ALS in aggregate, psychological health is, on average, poorer than
737 that of the population as a whole²²⁹. This has substantial implications as depression, hopelessness and
738 anxiety all associated with a poor QOL. Psychological interventions have been less well studied²³⁰ and
739 this warrants further attention.

740
741
742 QOL can affect the wishes for care of patients with ALS at the end of their lives. In a study from the
743 Netherlands, 16.8% of patients with ALS chose physician-assisted death, common reasons for which
744 were hopelessness, loss of dignity, dependency on others and fatigue²¹⁵. Similarly, the decision for
745 euthanasia in patients with ALS in Washington State was driven by loss of autonomy, participation in
746 enjoyable activities and dignity²¹⁶. These studies do not prove poor QOL in these individuals, but they
747 do raise this as a concern. The quality of death in patients with ALS has been studied less
748 comprehensively. Death was perceived as peaceful by 88% to 98% of caregivers in Germany, the United
749 Kingdom, the United States and Canada^{217, 231}. However, caution must be used in interpreting grouped
750 statistics. Incompletely relieved symptoms such as coughing from mucus, restlessness, anxiety and
751 muscle cramps resulted in moderate to severe suffering in the last 24 hours of life in 8 of 171 patients
752 ²¹⁷.

753

754 **[H1] Outlook**

755

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

764

765 **[H2] Epidemiology**

766 We can expect that the numbers of patients with ALS will increase in the future ²¹⁸, and that population
767 differences in incidence and phenotype will be recognized. Better multidisciplinary care and an
768 improved understanding of interventions means that a patient diagnosed with ALS can expect to live
769 longer than previously. In addition, the development of new drugs to improve respiratory function or
770 directly affect the disease process are expected to improve survival.

771

772 **[H2] Pathophysiology**

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

784

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

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

799

800 **[H2] Diagnosis and prognosis**

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

807

808 **[H2] Management**

809

810 **[H3] Clinical Trials**

811 The validity of pre-clinical studies should be evaluated rigorously by evidence-based analyses, and
812 translation of new therapies to humans should be undertaken only if findings are robust and
813 reproducible. Moreover, as ALS is a human disease, testing safe candidate compounds without prior
814 testing in animal models could be undertaken. In this instance, careful phase I and 2 studies including
815 detailed pharmacokinetic studies with extensive dose-finding and toxicity studies will be needed. As

816 some previous ALS clinical trials failed due to faulty trial design, a detailed correlative analysis of drug
817 levels in serum and CSF should be undertaken in early phases trials, and all trials should include a
818 biomarker readout to confirm that the drug is reaching its target.

819
820 The failure of previous clinical trials for ALS could also result from disease heterogeneity. Methods to
821 stratify patients that have a shared pathobiology are urgently required, and in the absence of this, pre-
822 specified, post-hoc analyses should be used to identify potential responder groups. This is exemplified
823 by a recent successful Phase 3 trial of edaravone¹⁸⁴, as recruitment to this trial was based on a post-hoc
824 analysis to identify possible responders, and stringent recruitment criteria were used to provide a
825 clinically homogeneous population that were likely to respond to treatment.

826
827 **[H3] New Drugs**

828 An extensive pipeline of new therapeutics for ALS is available, and some of these drugs target known
829 mutations and pathogenetic pathways. Symptomatic therapies including tirazemptiv based on
830 improving respiratory function in patients with ALS are currently in Phase 3 trials and exciting Phase I
831 trials assessing the use of antisense oligonucleotides in *SOD1* and *C9orf72* [related ALS are underway. In
832 the future, treatments are likely to be targeted at specific subgroups of patients and biomarkers that are
833 personalized to the individual disease subtype and have been developed from patient subcohorts that
834 have been extensively phenotyped and stratified using genomics, transcriptomics, metabolomics and
835 advanced imaging and signal analysis.

836
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838

839 **Display items**

840

841 **Box 1. Mechanisms of SOD1 toxicity in cellular and rodent models**

842 Transgenic mice with mutations in *SOD1* (encoding superoxide dismutase, SOD1) can be used to study
843 ALS pathophysiology. These mice over-express mutant SOD1 and many have an aggressive disease
844 course over approximately 80-90 days. However, they display quite well clinical and pathological
845 features similar to human ALS.

846 *SOD1* mutations can drive neurotoxicity in several ways, including protein misfolding [proteasome
847 impairment, excitotoxicity, oxidative stress, ER stress, impaired axonal transport, axonopathy,
848 inflammation, altered RNA processing and mitochondrial dysfunction].²³² Other mechanisms of SOD1-
849 related neurotoxicity have recently emerged and have gained interest. SOD1 can acts as a transcription
850 factor for genes involved in resistance to oxidative stress and repair of oxidative damage ²³³. RNA
851 oxidation is emerging as a prominent pathological outcome of generalized oxidative stress in the cell
852 with increasing importance in neurodegeneration Astrocytes and oligodendrocytes reprogrammed from
853 fibroblasts of patient with SOD1 mutations have been shown to induce hyperexcitability and cell death
854 in healthy control motor neurons. Glial toxicity is mediated through both contact (lactate independent)
855 and soluble mechanisms and is rescued by SOD1 knockdown using short hairpin RNA in glia derived
856 from patients with AOS1-related familial ALS, but also in glia derived from patients with sporadic ALS
857 without SOD1 mutation ¹¹³. Wild-type and mutant SOD1 proteins form insoluble intraneuronal fibrils,
858 which aggregate with increased propensity in the mutant form. A prion-like transmission of mutant
859 SOD1 fibrils can seed wild-type SOD1 protein aggregation in neighbouring neurons and propagate
860 neuronal injury²³⁴.

861 **Box 2. El Escorial criteria.**

862

863

864 **Figure 1. Clinical manifestations of ALS**

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

868 **Figure 2. Histopathology of ALS.**

869 a) Normal localization of TDP-43 in the nucleus (black arrow head), and aberrant localisation in a
870 diseased neuron with loss of nuclear expression and a 'skein-like' inclusion in the cytoplasm (black
871 arrow). b) Normal motor neuron (black arrow) and a hyaline conglomerate inclusion that stains for
872 SMI31 (black arrow head) in a patient with ALS caused by a *SOD1* mutation. c) TDP-43-negative, p62
873 positive dipeptide repeat inclusions with a 'stellate' morphology in the pyramidal cells of CA4 (black
874 arrow) and granule cells of the dentate fascia (black arrow head) in the hippocampus of a patient with
875 ALS caused by a mutation in *C9orf72*. d) The spinal cord ventral horn of a patient with ALS and a
876 healthy individual (e) showing a depleted numbers of motor neurons in ALS (arrows). F) CD68 (a
877 microglial marker) immunohistochemistry shows marked microglial reactivity in the lateral tracts (black
878 arrow) and ventral horns (black arrowhead), with no labelling in the dorsal columns (white arrow).

879
880

881 **Figure 3. Pathophysiology of ALS .**

882 Mutations in several amyotrophic lateral sclerosis (ALS) causative genes can exert motor neuronal injury
883 through more than one pathophysiological mechanism, although these mechanisms are often
884 interlinked. *SOD1* is the longest studied gene implicated in ALS and has been linked to the most
885 pathophysiological mechanisms, although the effects of mutations in *ALS3* and *ALS7* are still unknown.
886 Aberrant RNA metabolism and impaired protein homeostasis are predominant factors linking multiple
887 ALS causative genes to neuronal injury. Mitochondrial dysfunction can arise from a mutation in
888 *CHCHD10* and from secondary respiratory chain deficiencies that arise from protein aggregates
889 generated in the presence of other ALS genetic mutations Both cases lead to an increase in oxidative
890 stress, which puts further stress on an already impaired protein homeostasis system. Other mechanisms
891 of ALS can directly alter neuronal function (such as nuclear export, impaired DNA repair, dysregulated
892 vesicle transport and axon dysfunction) and the function of non-neuronal glial cells. The interplay of
893 mechanisms is indicated by arrows.

894

895 **Figure 4. Staging systems for ALS.**

896 The King's staging system is based on the number of body regions affected by ALS and the presence of
897 respiratory or nutritional failure ¹⁶². The Milano-Torino staging (MITOS) system is based on the ALS
898 functional rating scale (ALSFRS-R), a 48 point clinical measurement scale that records changes in bulbar,
899 gross motor, fine motor and respiratory parameters ¹⁶³. These staging systems do not incorporate
900 cognitive or behavioural changes. The King's staging system is sensitive to early changes in ALS, but the
901 sensitivity of the MITOS scale is greater in the later stages of disease ^{164, 165}.

902

903

904 **Figure 5. Factors affecting QOL in patients with ALS.**

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

909

910

911 Table 1. Genes implicated in ALS.

Gene locus	Gene (protein)	Inheritance	Implicated disease mechanisms	References
ALS1	<i>SOD1</i> (Superoxide dismutase 1)	AD/AR	Oxidative stress	235, 236
ALS2	<i>ALS2</i> (Alsin)	AR	Endosomal trafficking	237, 238
ALS3	Unknown	AD	Unknown	239
ALS4	<i>SETX</i> (Senataxin)	AD	RNA metabolism	240
ALS5	Unknown	AR	DNA damage repair, axon growth	241
ALS6	<i>FUS/TLS</i> (Fused in sarcoma/translated in liposarcoma)	AD/AR	RNA metabolism	242, 243
ALS7	Unknown	AD	Unknown	244
ALS8	<i>VAPB</i> (Vesicle associated membrane protein (<i>VAMP</i>) – associated protein B)[Au: should this be split up into two rows? Have <i>VAMP</i> and <i>VAPB</i> both been implicated in ALS?]	AD	ER stress	42
ALS9	<i>ANG</i> (Angiogenin)	AD	RNA metabolism	245
ALS10	<i>TARDBP</i> (TAR DNA binding protein)	AD	RNA metabolism	27, 246
ALS11	<i>FIG4</i> (Polyphosphoinositide 5-phosphatase)	AD	Endosomal trafficking	247
ALS12	<i>OPTN</i> (Optineurin)	AD/AR	Autophagy	248
ALS13	<i>ATXN2</i> (Ataxin 2)	AD	RNA metabolism	249
ALS14	<i>VCP</i> (Valosin-containing protein)	AD	Autophagy	36
ALS15	<i>UBQLN2</i> (Ubiquilin 2)	XD	UPS, autophagy	34
ALS16	<i>SIGMAR1</i> (Sigma non-opioid intracellular receptor 1)	AD	UPS, autophagy	250, 251
ALS17	<i>CHMP2B</i> (Charged multivesicular body protein 2B)	AD	Endosomal trafficking	252
ALS18	<i>PFN1</i> (Profilin 1)	AD	Cytoskeleton	97
ALS19	<i>ERBB4</i> (V-erb-b2 avian erythroblastic leukaemia viral oncogene homolog 4)	AD	Neuronal development	253
ALS20	<i>HNRNPA1</i> (Heterogeneous)	AD	RNA metabolism	82

nuclear ribonucleoprotein
A1)

ALS21	<i>MATR3</i> (Matrin 3)	AD	RNA metabolism	83
ALS22	<i>TUBA4A</i> (Tubulin alpha-4A)	AD	Cytoskeleton	102
ALS-FTD1	<i>C9orf72</i> (Chromosome 9 open reading frame 72)	AD	RNA metabolism, autophagy	5, 6
ALS-FTD2	<i>CHCHD10</i> (Coiled-coil-helix-coiled-coil-helix domain containing 10)	AD	Mitochondrial maintenance	255
ALS-FTD3	<i>SQSTM1</i> (Sequestosome 1)	AD	Autophagy	256
ALS-FTD4	TBK1 (TANK-binding kinase 1)			53, 54

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

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