Appendix for:

Amyotrophic lateral sclerosis

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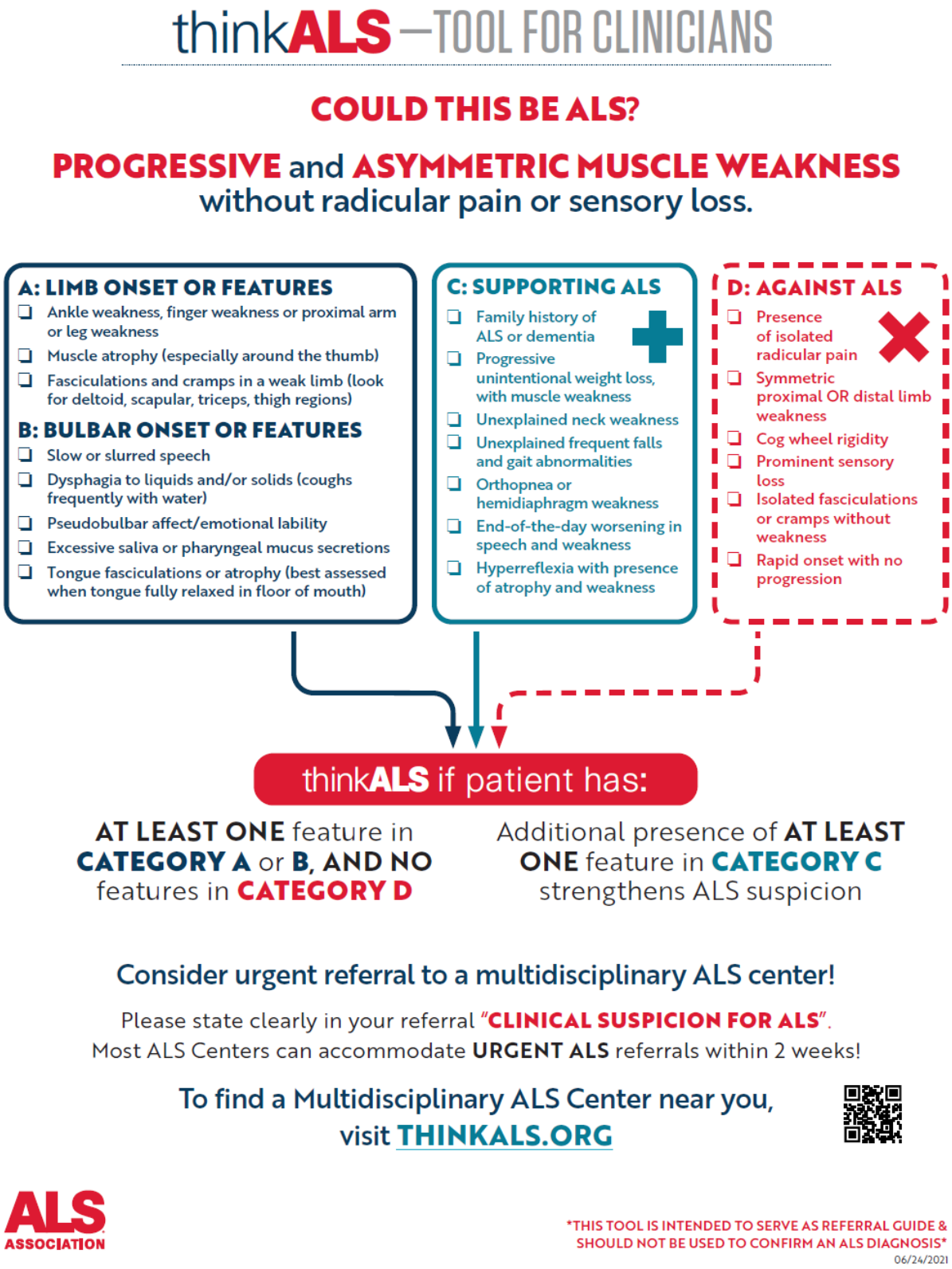
Appendix Figure 1. thinkALS tool for clinicians to encourage earlier neurological referral[1]



Appendix Table 1. ALS phenotypic heterogeneity.

Most common ALS presentations are bulbar and classical limb onset (cervical, lumbar), top parts of the table. Bulbar onset also occurs in less common nonclassical subsets, progressive bulbar palsy and pseudobulbar palsy. Additional less common ALS presentations are flail leg, pyramidal, flail arm, primary lateral sclerosis,* progressive muscular atrophy,* respiratory onset, hemiplegic, and cachexia, bottom part of the table.

ADM, abductor digiti minimi; APB, abductor pollicis brevis; FDI, first dorsal interosseous; LMN, lower motor neuron; ND, not determined in referenced study; TFL, tensor fascia lata; UMN, upper motor neuron.

* This review considers primary lateral sclerosis and progressive muscular atrophy are on the spectra of ALS phenotypes, although they may also be considered as separate clinical entities.

MOST COMMON

Phenotype	Frequency[2,3]	Presenting symptoms	Range of symptoms	Notes	Examination
Bulbar					
Bulbar onset	30.3%	UMN + LMN signs; typically, dysarthria presents first, then dysphagia	Typically, progresses to limbs and respiratory function	Patients may describe a heavy or numb tongue; query trouble managing secretions; may have unexplained weight loss; typically benefit from earlier feeding tube placement versus limb onset; bulbar weakness may hamper pulmonary function testing, so a mask for testing may be more optimal	Dysarthria. Look for tongue fasciculations, atrophy, and slowness; look for brisk jaw jerk and changes in gag reflex
Pseudobulbar palsy (nonclassical)	ND	UMN > LMN bulbar ALS	Prominent bulbar features, which slowly spread to limbs	Female predominant, longer survival; initial symptoms restricted to bulbar segment for prolonged time period	Spastic dysarthria, brisk jaw jerk and gag reflex, lack of limb weakness and hyperreflexia, pseudobulbar affect

MOST COMMON, continued

Phenotype	Frequency[2,3]	Presenting symptoms	Range of symptoms	Notes	Examination
Classical spinal; UMN + LMN signs					
Cervical onset	34.2%	Hand weakness is typical onset; split hand with APB and FDI atrophy is unique to ALS	Progresses proximally in the arm of onset, then progresses to cervical segment on contralateral side and lumbar segment on ipsilateral side; respiratory symptoms typical	Patients will describe trouble with hand dexterity or grip; may report trouble lifting early in disease; carpal tunnel syndrome can be an early consideration, but lack of sensory symptoms should suggest ALS	Look for APB/FDI weakness and atrophy relative to ADM (split hand); note presence of hyperreflexia and abnormal reflexes, especially Hoffmann's, Tromner, and finger flexor reflexes
Lumbar onset		Foot drop is early presenting symptom	Progresses proximally in the leg of onset, then progresses to lumbar segment on contralateral side and cervical segment on ipsilateral side; respiratory symptoms anticipated	Patients will notice a change in their gait or a tendency to trip; symptoms may be blamed on poorly fitting shoes or aging; query for unexplained falls	Look for increased patellar and ankle reflexes; look for extensor plantar response; if toe extensors are weak, look for TFL contraction with plantar stimulation

LESS COMMON

Phenotype	Frequency[2,3]	Presenting symptoms	Range of symptoms	Notes	Examination
Flail leg	13.0%	LMN without UMN in distal leg muscles, which may be asymmetric	Does not spread or spreads very slowly compared to classical ALS	Initially restricted to legs	LMN weakness; UMN signs will often develop
Pyramidal	9.1%	UMN > LMN	Progression is slower than classical ALS	Like classical ALS, but UMN signs are predominant; however, LMN signs present, differentiating this from primary lateral sclerosis	Spasticity with pathologic reflexes with some LMN signs
Flail arm; may also be referred to as brachial amyotrophic diplegia	5.5%	LMN much greater than UMN in upper limbs with proximal > distal involvement	Profound weakness in upper limbs with eventual spread to other segments	Initially restricted to arms; more prominent in males	Degree of upper limb weakness may mask brisk reflexes, check pectoral responses
Primary lateral sclerosis	4.0%	Progressive UMN signs in the limbs or facial muscles	Typically, will spread to limbs and affect speaking and swallowing	May be a separate entity from ALS; new diagnostic criteria[4]	UMN signs in limbs and bulbar muscles
Progressive muscular atrophy	2.9%	Progressive LMN signs in the limbs	Spread like classical ALS	Male predominance	Absence of UMN signs
Respiratory onset	1.1%	UMN + LMN; progressive neuromuscular respiratory weakness	Spreads to other segments	Rapid disease	Assess diaphragm function
Hemiplegic (Mill's variant)	ND	Progressive UMN > LMN weakness on one side of body	Often begins in leg and spreads to ipsilateral arm	Patients may have protracted disease course	Ipsilateral UMN signs in early disease course
Cachexia	ND	Progressive unintentional weight loss	Spreads to other segments	Rapid disease	UMN and LMN findings in distribution consistent with ALS

Appendix Table 2. Criteria for diagnosing ALS.

Criteria for diagnosing ALS include the original El Escorial,[5] revised El Escorial (also called the Arlie House),[6] Awaji,[7] and most recently the Gold Coast.[8] Modified from Goutman 2017.[9] Neuropathologic exam constitutes muscle/nerve biopsy.

EDx, electrodiagnostic testing; LMN, lower motor neuron; UMN, upper motor neuron.

CRITERIA				
	El Escorial	**Revised El Escorial**	**Awaji**	**Gold Coast**
ALS diagnosis	Presence of: (**1**) LMN signs by clinical, EDx, or neuropathologic exam; (**2**) UMN signs by clinical exam; and (**3**) progressive spread of signs within a region or to other regions, while excluding (**1**) other disease processes explaining LMN and/or UMN signs by EDx, (**2**) other diseases explaining the observed clinical and EDx signs by neuroimaging.	(**A**) Presence of: (**A:1**) LMN signs by clinical, EDx, or neuropathologic exam (**A:2**) UMN signs by clinical examination, and (**A:3**) progressive symptom or sign spread within a region or to other regions, as determined by history or exam, With: (**B**) Absence of: (**B:1**) EDx or pathological evidence of other diseases explaining LMN and/or UMN signs, and (**B:2**) neuroimaging evidence of other diseases explaining the observed clinical and EDx signs.	(**A**) Presence of: (**1**) LMN signs by clinical, EDx or neuropathological exam, (**2**) UMN signs by clinical exam, and (**3**) progressive symptom or sign spread within a region or to other regions, as determined by history, physical exam, or EDx tests. (**B**) Absence of: (**1**) EDx or pathological evidence of other diseases explaining LMN and/or UMN signs, and (**2**) neuroimaging evidence of other diseases explaining the observed clinical and EDx signs.	(**1**) Progressive motor dysfunction documented by history or repeated clinical exam, preceded by normal motor function, and (**2**) presence of UMN and LMN dysfunction in at least one body region, with UMN and LMN dysfunction present in the same body region if only one body region is involved, or LMN dysfunction in at least two body regions, and (**3**) investigations excluding other diseases.

DIAGNOSTIC CATEGORIES [a]				
	El Escorial	**Revised El Escorial**	**Awaji**	**Gold Coast**
Clinically definite	Clinical evidence of: (**1**) UMN + LMN signs in the bulbar and two spinal regions ***OR*** (**2**) UMN + LMN signs in three spinal regions	Same as the original El Escorial	LMN signs defined by clinical ***OR*** EDx evidence; otherwise, same as the El Escorial	None, criteria lead to a diagnosis of “ALS” or “no ALS”
Clinically probable	Clinical evidence of: UMN + LMN signs in at least two regions with UMN signs rostral to LMN signs	Same as the original El Escorial	LMN signs are defined by clinical ***OR*** EDx evidence; otherwise, same as the El Escorial	
Clinically probable – laboratory supported	Not included	Clinical evidence of (**1**) UMN + LMN signs in one region or UMN signs alone in one region ***AND*** (**2**) LMN by EDx criteria in at least two regions	Not included	
Clinically possible	Clinical evidence of: (**1**) UMN + LMN in one region ***OR*** (**2**) UMN signs in two or more regions ***OR*** (**3**) LMN signs are rostral to UMN signs	Same as the original El Escorial	LMN signs are defined by clinical ***OR*** EDx evidence; otherwise, same as the El Escorial	
Clinically suspected	Clinical evidence of: LMN signs in two or more regions	Deleted from the original El Escorial	Not included	

ELECTRODIAGNOSTIC FEATURES [b]				
	El Escorial	**Revised El Escorial**	**Awaji**	**Gold Coast**
Active denervation	Fibrillation potentials	Fibrillation potentials or positive sharp wave	Same; additionally, fasciculation potentials in muscles with chronic denervation	Fibrillation potentials, positive sharp waves, or (complex) fasciculation potentials
Chronic denervation	Reduced recruitment, large motor unit action potentials	Large motor unit potentials, lower interference pattern with firing rate >10 Hz, or unstable motor unit potentials	Same	Large motor unit potentials with increased duration and/or amplitude and polyphasia
SENSITIVITY AND SPECIFICITY				
	El Escorial	**Revised El Escorial**	**Awaji**	**Gold Coast**
Sensitivity	-	45 to 88.6%[10]	57 to 90.3%[10,11]	88.2 to 96.6%[11-13]
Specificity	-	96.2 to 100%[10]	95.5 to 100%[10,11]	17.4 to 88.5%[11-13]

Appendix Table 3. Revised Strong criteria for diagnosing cognitive and behavioral impairment in ALS.

The revised Strong criteria[14] are used to diagnose cognitive and behavioral dysfunction in ALS patients not meeting FTD criteria. Strong criteria define patients with cognitive dysfunction as "ALS cognitive impairment" (ALSci), behavioral problems as "ALS behavioral impairment" (ALSbi), and both cognitive and behavioral dysfunction as "ALS with combined cognitive and behavioral impairment" (ALScbi). FTD criteria include Neary[15] or Rascovsky[16] criteria.

ALSci	ALSbi	ALScbi
Relies on evidence of executive dysfunction (including social cognition), language dysfunction, or both combined. Executive dysfunction defined as: (**1**) Valid verbal fluency (letter) deficits, which must control motor and/or speech deficits,[17] ***AND/OR*** (**2**) Dysfunction in two other non-overlapping executive function measures which may include social cognition. Language dysfunction defined as: (**1**) Dysfunction in two non-overlapping tests, which could include pragmatic function.	Defined by: (**1**) Presence of apathy with or without other behavioral changes, ***AND/OR*** (**2**) Presence of two or more of the following behavioral symptoms: (**a**) disinhibition, (**b**) loss of sympathy and empathy, (**c**) perseverative, stereotyped, or compulsive behavior, (**d**) dietary change, (**e**) loss of insight, (**f**) psychotic symptoms (***e.g.***, somatic delusions, hallucinations, irrational beliefs). Apathy with symptoms (a) to (d) constitute the criteria for behavioral variant FTD.[16]	New classification (since the original Strong criteria) of patients fulfilling criteria for both ALSci and ALSbi.

Appendix Table 4. Summary of ALS genotype-phenotype associations and overlap with other diseases.

Adapted, with permission, from Goutman et al. The Lancet Neurology, 2022.[18] There is uncertainty in the relevance of some identified genes to ALS, which require further confirmation and replication efforts.[19]

Gene	Genetic effect	%FALS	%SALS	Associated clinical ALS phenotype	Overlap with other diseases
ALS2	Autosomal recessive	<1	<1	Slowly progressive, infantile and juvenile mainly affecting UMN, PLS	HSP
ANG	Autosomal dominant Risk factor	<1	<1	Typical, bulbar-onset tendency, FTD	
ANXA11	Autosomal dominant	~1	~1·7	ND	Autoimmune, sarcoidosis
ATXN2	Autosomal dominant Risk factor	<1	<1	Typical	SCA
C9orf72	Autosomal dominant	40	7	Typical, FTD	Huntington disease phenocopy, Parkinsonism, essential tremor, myoclonus
C21orf2	ND	<1	<1	Typical, FTD	
CCNF	Autosomal dominant	~1-3·3	<1	Typical, FTD, PLS	
CHCHD10	Autosomal dominant	<1	<1	Typical, FTD	Cerebellar ataxia, myopathy
CHMP2B	Autosomal dominant	<1	<1	Typical, PMA	FTD
DCTN1	Autosomal dominant Risk factor	<1	<1	Slowly progressive juvenile	Perry syndrome (Parkinsonism)
DNAJC7	ND	<1	<1	ND	
ELP3	Allelic	<1	<1	Typical	
FUS	Autosomal dominant Autosomal recessive *de novo*	4	1	Typical or atypical, FTD, dementia; juvenile, adult onset	Essential tremor*
GLT8D1	Autosomal dominant	<1	<1	Typical, shorter and longer survival	Schizophrenia
GRN	Autosomal dominant Modifier	<1	<1	Earlier onset, shorter survival	FTD, FTLD, DLB*

Gene	Genetic effect	%FALS	%SALS	Associated clinical ALS phenotype	Overlap with other diseases
HNRNPA1	Autosomal dominant *de novo* Risk factor	<1	<1	Typical, cognitive impairment	IBM
HNRNPA2B1	Autosomal dominant Risk factor	<1	<1	Typical, cognitive impairment	IBM
KIF5A	Autosomal dominant	~0·5-3	<1	Earlier onset, longer survival	CMT2, PPMS phenocopy*, SPG10
LGALSL	ND	<1	<1	Earlier onset, typical	
MATR3	Autosomal dominant	<1	<1	Slowly progressive typical or atypical, FTD, myopathy	Distal myopathy
NEFH	Autosomal dominant Risk factor	<1	<1	Typical	CMT2*
NEK1	ND	~1-2	<1	ND	
OPTN	Autosomal dominant Autosomal recessive	<1	<1	Slowly progressive atypical	Open-angle glaucoma, Paget's
PFN1	Autosomal dominant	<1	<1	Typical	
SETX	Autosomal dominant	<1	<1	Slowly progressive juvenile	SCA, progressive motor neuropathy
SPG11	Autosomal recessive	<1	<1	Slowly progressive juvenile, mainly affecting UMN	HSP
SOD1	Autosomal dominant Autosomal recessive *de novo*	12	1-2	Prominent LMN, cognitive impairment very rare	
SQSTM1	Autosomal dominant	~1	<1	Typical	Paget's, FTD, DLB*
TARDBP	Autosomal dominant Autosomal recessive *de novo*	4	1	Typical, FTD	Supranuclear gaze palsy
TBK1	Autosomal dominant *de novo*	~3	<1	Typical, FTD	FTLD, DLB*
TIA1	Autosomal dominant	~2·2	<1	FTD	DLB*
TUBA4A	Autosomal dominant	<1	<1	Typical, FTD	

Gene	Genetic effect	%FALS	%SALS	Associated clinical ALS phenotype	Overlap with other diseases
UBQLN2	X-linked autosomal dominant	<1	<1	Typical; juvenile, adult onset, FTD	FTD*
VAPB	Autosomal dominant	<1	<1	Typical or atypical	SMA, essential tremor
VCP	Autosomal dominant *de novo*	1	1	Typical, FTD	Inclusion body myositis with Paget's disease, Parkinsonism, SMD, dropped head syndrome

ALS2, alsin Rho guanine nucleotide exchange factor ALS2; *ANG*, angiogenin; *ANXA11*, annexin A11; *ATXN2*, ataxin 2; *C9orf72*, chromosome 9 open reading frame 72; *C21orf2*, chromosome 21 open reading frame 2; *CCNF*, cyclin F; *CHCHD10*, coiled-coil-helix-coiled-coil-helix domain containing 10; *CHMP2B*, charged multivesicular body protein 2B; CMT, Charcot-Marie-Tooth; *DCTN1*, dynactin subunit 1; *DNAJC7*, DnaJ homolog subfamily C member 7; DLB, dementia with Lewy bodies; *ELP3*, elongator acetyltransferase complex subunit 3; FALS, familial ALS; FTD, frontotemporal dementia; FTLD; frontotemporal lobar degeneration; *FUS*, Fused in Sarcoma; *GLT8D1*, glycosyltransferase 8 domain containing 1; *HNRNPA1*, heterogeneous nuclear ribonucleoprotein A1; *HNRNPA2B1*, heterogeneous nuclear ribonucleoprotein A2/B1; HSP, hereditary spastic paraparesis; IBM, inclusion body myopathy; *KIF5A*, kinesin family member 5A; *LGALSL*, galectin-like; LMN, lower motor neuron; *MATR3*, matrin 3; ND, not determined; NCP, nucleocytoplasmic transport; *NEFH*, neurofilament heavy chain; *NEK1*, NIMA (never in mitosis gene a)-related kinase 1; *OPTN*, optineurin; *PFN1*, profilin 1; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; PPMS, primary progressive multiple sclerosis; SCA, spinocerebellar ataxia; SPG10, hereditary spastic paraplegia; *SPG11*, SPG11 vesicle trafficking associated, spatacsin; SALS, sporadic ALS; *SETX*, senataxin; SMA, spinal muscular atrophy; SMD, scapuloperoneal muscular dystrophy; *SOD1*, superoxide dismutase 1; *SQSTM1*, sequestosome 1; *TARDBP*, TAR DNA binding protein; *TBK1*, TANK-binding kinase 1; *TIA1*, TIA-1 cytotoxic granule-associated RNA binding protein; *TUBA4A*, tubulin alpha 4a; *UBQLN2*, ubiquilin 2; UMN, upper motor neuron; *VAPB*, vesicle-associated membrane protein-associated protein B and C; *VCP*, valosin-containing protein.

*Findings limited to a few patients.

Appendix Table 5. Summary of most common potential ALS mimics relevant to differential diagnosis[20-22]

Abbreviations: cN1A, cytosolic 5'-nucleotidase 1A; CIDP, chronic inflammatory demyelinating polyneuropathy; CSF, cerebrospinal fluid; EDx, electrodiagnostic testing; EMG, electromyography; GM, ganglioside-monosialic acid; LMN, lower motor neuron; LRP4, low density lipoprotein receptor-related protein 4; MuSK, muscle specific receptor tyrosine kinase; UMN, upper motor neuron.

Disease	Pattern of weakness	Differentiating features	Diagnostic evaluation
Cervical stenosis with cervical radiculopathy	LMN at level of lesion and UMN caudal	Radicular pain, Lhermitte's sign, LMN symptoms isolated to level of compression, lack of UMN signs rostral to lesion	MRI spinal cord
Hexosaminidase A deficiency (Tay Sachs disease)	UMN and LMN	Initially proximal weakness and cramps suggesting pure LMN disorder; may develop spinocerebellar symptoms, cerebellar, psychosis, dystonia	Hexosaminidase A activity testing, complex repetitive discharges on EMG
Hyperparathyroidism	UMN and LMN	Extremity/abdominal pain, headache, paresthesia, fatigue, nausea, anorexia; preserved reflexes in weak limb may suggest UMN sign	Serum calcium, parathyroid hormone
Hereditary spastic paraparesis (HSP)	UMN	Family history, progressive weakness and spasticity in legs, early sphincter involvement, lack of bulbar involvement	Genetic testing
Primary progressive multiple sclerosis (PPMS)	UMN	Progressive symptoms, often with sensory involvement	MRI brain/spinal cord, CSF evaluation
Axonal motor predominant CIDP	LMN	Symmetric weakness, sensory symptoms, absent reflexes	Nerve conduction studies, CSF testing
Cramp-fasciculation syndrome (CFS)	Normal exam or fasciculations	No history of weakness	EDx
Hirayama's disease	Restricted LMN	Restriction of weakness to one limb, typically hand, usually young males	MRI cervical cord

Disease	Pattern of weakness	Differentiating features	Diagnostic evaluation
Inclusion body myositis (IBM)	LMN	Finger flexor and knee extensor weakness	Anti-cN1A antibodies, muscle biopsy
Multifocal motor neuropathy (MMN)	LMN	Distal and asymmetric weakness, slowly progressive, wrist drop, finger drop, lack of respiratory/bulbar involvement	Motor conduction block on nerve conduction studies, anti-GM1 ganglioside antibodies
Myasthenia gravis (MG)	LMN	Ptosis, diplopia, proximal weakness, fatigability	Acetylcholine receptor, MuSK, LRP4 antibodies, decrement on motor nerve conduction studies, increased jitter in single-fiber EMG
Neuralgic amyotrophy	LMN	Pain prior to presentation of weakness, weakness often involves specific pattern of nerves, respiratory involvement rare	Electrodiagnostic evaluation
Spinobulbar muscular atrophy (Kennedy's disease)	LMN	Males, proximal weakness, tremor, cramps, fasciculations (especially facial), gynecomastia, endocrine disorders (infertility, diabetes)	Gene testing: Androgen receptor

Appendix Table 6. ALS environmental studies.

English language studies of primary literature published in the past 5 years were identified with the search terms of ALS combined with environment, exposure, cluster, metals, pesticides, and pollutant and were selected to show the range of exposure assessments and based on population size, publication impact, and use of novel techniques.

Risk Factor	Study Cohort	Reference
Blood cadmium, lead, zinc	European Prospective Investigation into Cancer and Nutrition cohort	**Peters**[23]
Occupational factors		
Diesel exhaust (n males)	Danish National Patient Registry	**Dickerson**[24]
Agricultural work	Italian case-control study	**Filippini**[25]
Paint remover and thinner exposure	Italian case-control study	**Filippini**[25]
Metal exposure	Italian case-control study	**Filippini**[25]
Formaldehyde exposure	Danish National Patient Registry	**Seals**[26]
Silica dust	Euro-MOTOR cohort	**Visser**[27]
Organic dust	Euro-MOTOR cohort	**Visser**[27]
Blue-collar work including craft and related trades occupations	Malta case-control study	**Farrugia Wismayer**[28]
Military service	ALS prevalence in US military	**Sagiraju**[29]
Residential factors		
Private well drinking water	Italian case-control study	**Filippini**[30]
Herbicide gardening use	Italian case-control study	**Filippini**[30]
Living near a water body	Italian case-control study	**Filippini**[25]
Air pollution including $PM_{2.5}$, PM_{10}, CO	Korean case-control study	**Myung**[31]
Traffic-related air pollution	Netherlands, European Study of Cohorts for Air Pollution Effects	**Seelen**[32]

Proximity to agricultural crops	Italian case-control study	**Vinceti**[33]
Physical activity	UK biobank	**Julian**[34]
Trauma		
Trauma & Head Trauma	Italian case-control study	**Filippini**[30]
Traumatic injury	European Amyotrophic lateral sclerosis cohort	**Pupillo**[35]
Electric shock	Italian case-control study	**Filippini**[30]
Extremely low-frequency magnetic fields and electric shocks	European Multidisciplinary ALS Network	**Peters**[36]
Electromagnetic field exposure	Italian case-control	**Filippini**[25]
Extremely low-frequency magnetic fields	European Multidisciplinary ALS Network Identification	**Peters**[36]
Extremely low-frequency magnetic fields	Pooled analysis of multiple cohorts	**Baaken**[37]

Appendix Table 7. List of current ALS clinical trials for genetic therapies, antibodies, immune-targeting, and stem cell therapies.

All trials meeting the search criteria “amyotrophic lateral sclerosis” on the clinicaltrials.gov website and involving gene therapies, antibodies, immune-targeting agents, and stem cells are included in the table. MoA, mechanism of action.

Gene therapy trials, arranged alphabetically by gene

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT04494256	Phase 1	BIIB105	ASO against *ATXN2*	Intrathecal	ALS with or without poly-CAG *ATXN2* expansions	Safety, tolerability, PK	Recruiting	Canada Netherlands USA
NCT04931862	Phase1/2	WVE-004	ASO against *C9ORF72* mutation	Intrathecal	*C9ORF72*-associated ALS or FTD	Safety	Recruiting	Australia Canada Ireland Netherlands UK
NCT04931862	Phase 1/2	WVE-004	ASO against *C9ORF72* mutation	Intrathecal	*C9ORF72*-associated ALS or FTD	Safety	Recruiting	Australia Canada Ireland Netherlands UK
NCT04288856	Phase 1	BIIB078	ASO against *C9ORF72* mutation	Intrathecal	*C9ORF72*-associated ALS	Long-term Safety, tolerability, PK, effect on disease progression	Active not recruiting	Canada Netherlands Switzerland UK USA
NCT04768972	Phase 3	ION363	ASO against *FUS* mutation	Intrathecal	FUS-ALS	Safety, efficacy, PD, PK	Recruiting	USA
NCT04632225	Phase 2	Engensis	HGF gene plasmid	Intramuscular	Sporadic and familial ALS	Safety	Recruiting	Korea USA

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT02623699	Phase 3	BIIB067	ASO against *SOD1* mutation	Intrathecal	Harboring *SOD1* mutation	Safety, efficacy, tolerability, PD, PK	Completed	Canada Europe Korea Japan UK USA
NCT04856982	Phase 3	BIIB067	ASO against *SOD1* mutation	Intrathecal	Presymptomatic adult mutant *SOD1* carriers with elevated NfL	Percentage of participants developing clinically manifest ALS	Recruiting	Canada Europe Korea Japan UK USA
NCT04972487	Expanded access program	BIIB067	ASO against *SOD1* mutation	Intrathecal	Rapidly progressive ALS	Efficacy	Available	Not applicable

Antibody trials with non-immune targets, arranged alphabetically by protein target

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT05053035	Phase 2	AL001	Monoclonal, elevate progranulin	Intravenous	*C9ORF72*-associated ALS	Safety, tolerability, PD, PK	Recruiting	USA
NCT05039099	Phase 2	AP-101	Monoclonal, against SOD1 protein	Intravenous	Sporadic ALS, mutant *SOD1* familial ALS	Safety, tolerability, PD markers, PK	Recruiting	Canada USA

Immune-targeting trials, including immune-targeting antibodies, arranged alphabetically by agent

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT05039268	Phase 2	3K3A-APC protein	Reduced microglia activation	Intravenous	Sporadic and familial ALS	Safety, change in microglial activation in motor cortex of [^{18}F]-FEMPA PET	Recruiting	Australia
NCT04569435	Phase 2	ANX005	Monoclonal, against C1q protein	Intravenous	Sporadic and familial ALS	Safety	Recruiting	Canada USA
NCT04322149	Phase 2a	AT-1501	Monoclonal, against CD40L protein	Intravenous	Possible, LS-probable, probable, or definite ALS	Safety, tolerability	Recruiting	Canada USA
NCT04066244	Phase 2	BLZ945	Reduce microglia activation	Oral	Sporadic and familial ALS	Change from baseline in volume of distribution in different brain regions of [^{11}C]-PBR28 PET	Recruiting	Finland Sweden USA
NCT05006352	Phase 1	DNL343	Small molecule EIF2B agonist	Oral	Sporadic and familial ALS	Safety, PD, PK	Recruiting	Netherlands USA
NCT03766321	Not applicable	Fecal microbiota transplant	Immuno-modulating	Fecal microbiota transplant	Sporadic and familial ALS	Safety, tolerability	Recruiting	Italy
NCT04057898	Phase 2/3	Ibudilast (MN-166)	Small molecule inflammation inhibitor	Oral	Sporadic and familial ALS	ALSFRS-R score	Recruiting	Canada USA
NCT03039673	Phase 2	IL-2	Enhance Tregs	Subcutaneous	Initial ALS	Survival	Completed under analysis	France UK

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT04952155	Phase 2	IL-2	Enhance Tregs	Subcutaneous	Immune-mediated ALS syndrome	ALSFRS-R score	Recruiting	China
NCT03652805	Phase 1/2	IPL-344	PI3K-Akt signaling agonist	Intravenous	Sporadic and familial ALS	Safety, tolerability	Recruiting	Israel
NCT03755167	Phase 2	IPL-344	PI3K-Akt signaling agonist	Intravenous	Sporadic and familial ALS	Safety, tolerability	Recruiting	Israel
NCT03127267	Phase 3	Masitinib	Immuno-modulating, targets mast cells and microglia	Oral	Fast progressors	ALSFRS-R score	Recruiting	Germany USA
NCT04579666	Phase 2	Pegcetacoplan	C3 inhibitor	Subcutaneous	Sporadic and familial ALS	Combined assessment of function and survival	Recruiting	Australia Europe Japan UK USA
NCT04248465	Phase 3	Ravulizumab	Monoclonal, against C5 protein	Intravenous	Sporadic and familial ALS	ALSFRS-R change from baseline	Terminated	Canada Europe UK USA
NCT03456882	Phase 2	RNS60	Enhance Tregs, activate protective astrocytes and microglia	Intravenous, inhalation	Sporadic and familial ALS	PD biomarkers	Terminated	Italy
NCT02988297	Phase 2	RNS60	Enhance Tregs, activate protective astrocytes and microglia	Inhalation	Sporadic and familial ALS	ALSFRS-R score	Not yet recruiting	USA
NCT04326283	Phase 1/2	Trametinib (SNR1611)	MEK inhibitor	Oral	Sporadic and familial ALS	Safety, tolerability	Recruiting	Korea

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT04220190	Phase 1/2	Autologous hybrid Treg/Th2 cells (RAPA-501)	Immuno-modulating	Intravenous	Sporadic and familial ALS	Safety	Recruiting	USA
NCT04055623	Phase 2a	Autologous Tregs with IL-2	Immuno-modulating	Intravenous	Sporadic and familial ALS	Change in peripheral Treg suppressive function	Active not recruiting	USA
NCT04436497	Phase 2/3 platform trial	Zilucoplan	C5 inhibitor	Subcutaneous	Sporadic and familial ALS	Change in disease severity over time by ALSFRS-R	Active not recruiting	USA

Stem cell trials, arranged alphabetically by trial phase

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT05003921	Phase 1	Allogeneic adult umbilical cord-derived MSCs	Multitarget	Intrathecal	Sporadic and familial ALS	Safety	Recruiting	Antigua Barbuda
NCT04651855	Phase1/2	MSCs isolated from Wharton's jelly	Multitarget	Intrathecal	Sporadic ALS	Safety	Recruiting	Poland
NCT02290886	Phase 1/2	Autologous MSCs	Multitarget	Intravenous	Sporadic ALS	Safety	Active not recruiting	Spain
NCT02478450	Phase 1/2	Human glial restricted progenitor cells	Multitarget	Unilateral cell transplant in lumbar or cervical spinal cord	Sporadic and familial ALS	Safety	Not yet recruiting	Unknown
NCT04849065	Phase 2	Autologous BMNCs	Multitarget	Intramuscular	Sporadic and familial ALS	Number and size of functional motor units	Not yet recruiting	Spain

Trial number	Trial type	Agent	MoA	Delivery mode	Patients	Outcome	Status	Location (alphabetically)
NCT03268603	Phase 2	Autologous adipose-derived MSCs	Multitarget	Intrathecal	Sporadic and familial ALS	Safety, change in ALSFRS-R slope	Recruiting	USA
NCT04745299	Phase 3	Autologous bone marrow-derived MSCs (Lenzumestrocel)	TGF-β1 and MCP1 levels	Intrathecal	Sporadic and familial ALS	Joint rank scores (CAFS)	Recruiting	Korea
NCT02795052	Not applicable	Autologous bone marrow-derived MSCs	Multitarget	Intravenous, intranasal	Have documented functional damage to the CNS or PNS	Activities of Daily Living (ADL)	Recruiting	United Arab Emirates USA

3K3A-APC, 3K3A-activated protein C; ALSFRS-R, ALS functional rating scale revised; ASO, antisense oligonucleotide; *ATXN2*, ataxin 2; BMNCs, bone marrow mononuclear cells; C1q, complement component 1q; C3, complement component 3; C5, complement component 5; C9ORF72, chromosome 9 open reading frame 72; CAFS, combined assessment of functional and survival; CNS, central nervous system; EIF2B, eukaryotic initiation factor 2B; FUS, Fused in Sarcoma; HGF, hepatocyte growth factor; IL-2, interleukin 2; LS-probable, laboratory supported probable; MCP1, monocyte chemoattractant protein 1; MEK, Mitogen-activated protein kinase kinase; MoA, mechanism of action; MSCs, mesenchymal stem cells; NfL, neurofilament; PD, pharmacodynamics; PK, pharmacokinetics; PNS, peripheral nervous system; SOD1, superoxide dismutase 1; Th2, type 2 helper T cells; TGF-β1, transforming growth factor beta 1; Tregs, regulatory T cells.

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