State of Hawaii
Department of Health
4348 Waialea Avenue #648
Honolulu, HI 96816

Medical Marijuana Registry

# PETITION TO ADD A DEBILITATING MEDICAL CONDITION IN 2017

## Instructions

1. ALL items on the form MUST be completed.

2. Petitions and any supporting documents may be submitted as follows:
   a. Email to: medicalmarijuana@doh.hawaii.gov before the close of business (4:30 PM) on **Friday, June 16, 2017**. Please use the subject line: Petition to Add New Condition. Note that the DOH will not make public any information that is protected pursuant to Chapter 92F, HRS, the Uniform Information Practices Act.

   b. Postal mail to: 4348 Waialae Avenue, #648, Honolulu, Hawaii 96816. Mailed petitions must be received by **June 16, 2017**.

   c. Hand delivered to: Kinau Hale at 1250 Punchbowl Street, Honolulu, Hawaii 96813 before the close of business (4:30 PM) on Friday, June 16, 2017. Hand delivered petitions must be left with the security guard and addressed to the Medical Marijuana Registry Program **ATTN: Petition to Add New Condition**.

3. For best results, complete and thorough petitions that include substantiated and reputable research have the best chance of succeeding. DOH recommends that you do the following for items #2 - #8 on the petition form:
   a. Please cite research, published evidence, or findings using the standard American Medical Association (AMA) format for each piece of research, published evidence, or findings that you reference in your submittal or at a minimum the following:

      Author's Name; Title of Article; Name of Publication; Date of Publication; Volume/Section/Chapter/Page/Line as applicable; and URL (if applicable).

   b. Please attach a PDF copy of the cited material to your submittal. These documents will NOT be returned.

   c. Please be sure to indicate the specific section, page(s), lines, etc., of the attachment that you want reviewed/considered as evidence.

4. To view a list of current conditions click here: Current Debilitating Medical Conditions

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**Petitioner Content:**

(1) State the specific medical condition or its treatment for which the petition is being made.

Amyotrophic lateral sclerosis (ALS).

(2) State the reason(s) why the medical condition or its treatment should be added to the list of qualifying debilitating medical conditions for which medical marijuana may be used. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Reason #1: Pre-clinical research has shown that the administration of Delta-9-THC in a mouse model for ALS can delay motor impairment and prolong survival (Reference #1, Page 38).

Reason #2: The mechanisms of pathogenesis in ALS, including excitotoxicity, inflammation, and oxidative stress, have been well characterized and support the medical use of Cannabis for this condition (Reference #2, Page 2311).

Reason #3: In addition to potentially delaying motor impairment and prolonging survival, the medical use of Cannabis also holds potential for relieving the disabling symptoms associated with ALS, such as pain, spasticity, wasting, respiratory failure, dysphagia, depression, and dysautonomia (Reference #3, page 267).

Reason #4: A survey of ALS patients found the medical use of Cannabis to be moderately effective at reducing symptoms of appetite loss, depression, pain, spasticity, and drooling (Reference #4, page 95).

Reason #5: With the potential for the medical use of Cannabis to delay the progression of ALS, It is medically inappropriate to wait for the debilitating symptoms of this disease to become manifest before allowing certification to occur. Once the diagnosis of ALS is made, the option to engage in the medical use of Cannabis should be made available immediately.

Reason #6: ALS is already a recognized debilitating condition in the following states/districts: AR, AZ, CA, DE, D.C., FL, GA, IL, ME, MA, MI, MN, ND, NH, NJ, NM, NY, OH, PA.

Reason #7: Cannabidiol (CBD), another component of Cannabis, has been shown to have anti-inflammatory, anti-oxidant, and neuro-protective properties that may be ideal for treating the neuro-toxicity associated with ALS (Reference #5, Pages 13S and 17S).

(3) Describe the extent to which the medical condition is generally accepted by the medical community as a valid, existing medical condition. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

ALS is an established diagnosis in the medical community, and well characterized in the scientific literature (Reference #6, Page 942).

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(4) Describe the symptoms and other physiological or psychological effects experienced by an individual suffering from the medical condition or its treatment and the extent to which these symptoms and physiological or psychological effects are debilitating. Note: "Debilitating" generally means impairing the ability of a person to accomplish activities of daily living. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Debilitating symptom #1: Muscle spasticity - caused by loss of motor neuron inhibition as motor neuron degeneration progresses, which, in addition to loss of muscle mass, leads to worsening immobility and exacerbation of Debilitating symptom #2 (Reference #7, page 350).

Debilitating symptom #2: Pain - experienced by a majority of ALS patients with significant suffering and decrease in quality of life, due to immobility which causes adhesive capsulitis, mechanical back pain, pressure sores, and neuropathic pain, in addition to the excruciating pain from muscle spasms that result from Debilitating symptom #1 (Reference #7, page 350).

Debilitating symptom #3: Loss of appetite - causes what is known as "ALS cachexia", which results in loss of body mass in excess of what would be expected from muscle atrophy and decreased calorie intake, further worsening immobility and the resulting pain issues described in Debilitating symptom #2 (Reference #7, page 351).

Debilitating symptom #4: Drooling - caused by difficulty controlling and swallowing saliva that is normally produced in the mouth, causing increased discomfort and the risk of aspiration pneumonia (Reference #7, page 351).

(5) If one or more treatments for the medical condition, rather than the condition itself, are alleged to be the cause of a person's suffering, describe the extent to which the treatments causing suffering are generally accepted by the medical community as valid treatments for the medical condition. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

N/a.

(6) Describe the availability of conventional medical therapies other than those that cause suffering to alleviate symptoms caused by the medical condition or its treatment. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Currently, the only FDA-approved drug treatment for ALS is Riluzole, which is believed to function by means of decreasing glutamate neuro-toxicity, and which has been shown to minimally prolong survival in clinical trials (Reference #8, Page 182). However, Riluzole only appears helpful in the early stages of the disease, with a favorable effect that was transient and lost in prolonged follow-up (Reference #9, page 262).

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(7) Describe the extent to which evidence supports a finding that the use of marijuana alleviates symptoms caused by the medical condition or its treatment. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

A survey of ALS patients found the medical use of Cannabis to be moderately effective at reducing symptoms of appetite loss, depression, pain, spasticity, and drooling (Reference #4, page 95).

(8) Provide any information, studies, or research reports regarding any beneficial or adverse effects from the use of marijuana in patients with the medical condition. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

See #7 above.

(9) Attach letters of support from physicians or other licensed health care professionals knowledgeable about the medical condition.

See attached letter of support from Gregory Carter, MD (Reference #10).

You MUST provide a Number and Name for each Attachment referenced above and provide a list of these attachments here. This way we can ensure that your petition was submitted with all of the applicable attachments:

REFERENCES:

(1) Raman C, McAllister S, Rizvi G, et al. Amytrophic lateral sclerosis: delayed progression in mice by treatment with a cannabinoid. ALS and other motor neuron disorders. 2004; 5: 33-39.

(2) Bilsland LG and Greensmith L. The endocannabinoid system in amyotrophic lateral sclerosis. Current Pharmaceutical Design. 2008; 14: 2306-2316.

(3) Carter GT and Rosen BS. Marijuana in the management of amyotrophic lateral sclerosis. Am J Hosp Palliat Care. 2001; 18(4): 264-270.

(4) Amtmann D, Weydt P, Johnson KL, et al. Survey of cannabis use in patients with amyotrophic lateral sclerosis. Am J Hosp Palliat Care. 2004; 21: 95-104.

(5) Mechoulam R, Parker LA, Gallily R. Cannabidiol: an overview of some pharmacological aspects. Journal of Clinical Pharmacology. 2002; 42: 11S-19S.

(6) Kiernan MC, Vucic S, Cheah BC, et al. Amyotrophic lateral sclerosis. Lancet. 2011; 377: 942-955.

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REFERENCES (cont.):

(7) Carter GT, Abood ME, Aggarwal SK, et al. Cannabis and amyotrophic lateral sclerosis: hypothetical and practical applications, and a call for clinical trials. Am J Hosp Palliat Care. 2010; 27(5): 347-356.

(8) Zarei S, Carr K, Reiley L, et al. A comprehensive review of amyotrophic lateral sclerosis. Surgical Neurology International. 2015; 6: 171-193.

(9) Zoccolella S, Beghi E, Palagano G, et al. Riluzole and amyotrophic lateral sclerosis survival: a population-based study in southern Italy. European Journal of Neurology. 2007; 14: 262-268.

(10) Letter of support from Greg Carter, MD.

# REFERENCE #1

ALS and other motor neuron disorders 2004 5, 33–39
 ISSN 1466-0822



# Amyotrophic lateral sclerosis: delayed disease progression in mice by treatment with a cannabinoid

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**Effective treatment for amyotrophic lateral sclerosis (ALS) remains elusive. Two of the primary hypotheses underlying motor neuron vulnerability are susceptibility to excitotoxicity and oxidative damage. There is rapidly emerging evidence that the cannabinoid receptor system has the potential to reduce both excitotoxic and oxidative cell damage. Here we report that treatment with $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) was effective if administered either before or after onset of signs in the ALS mouse model (hSOD$^{G93A}$ transgenic mice). Administration at the onset of tremors delayed motor impairment and prolonged survival in $\Delta^9$-THC treated mice when compared to vehicle controls. In addition, we present an improved method for the analysis of disease progression in the ALS mouse model. This logistic model provides an estimate of the age at which muscle endurance has declined by 50% with much greater accuracy than could be attained for any other measure of decline. *In vitro*, $\Delta^9$-THC was extremely effective at reducing oxidative damage in spinal cord cultures. Additionally, $\Delta^9$-THC is anti-excitotoxic *in vitro*. These cellular mechanisms may underlie the presumed neuroprotective effect in ALS. As $\Delta^9$-THC is well tolerated, it and other cannabinoids may prove to be novel therapeutic targets for the treatment of ALS.** (ALS 2004; 5: 33-39)

**Keywords:** amyotrophic lateral sclerosis – $\Delta^9$-THC – cannabinoid – anti-oxidant – anti-excitotoxicity – neuroprotection

## Introduction

Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative cause of adult death, after Alzheimer's disease and Parkinson's disease.[1] ALS results in the degeneration of motor neurons in the cortex, brainstem and spinal cord.[1,2] Most causes of ALS are presently unknown and several mechanisms of insult to motor neurons have been suggested.[3–5] Two of the primary theories underlying motor neuron vulnerability are susceptibility to excitotoxicity and oxidative damage.[4,5] Cannabinoid agonists have been reported to reduce both excitotoxic and oxidative cell damage.[6,7] $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), in particular, is anti-excitotoxic and antioxidant in cellular and animal models.[7–10] This potentially synergistic action of a single compound may lead to an improved therapeutic effect as compared to conventional glutamate antagonists or antioxidants. Moreover, cannabinoid agonists modulate tremor and spasticity in a mouse model of multiple sclerosis.[11,12] Furthermore, ALS patients taking Marinol report a lessening of spasticity.[13]

Mutations in Cu/Zn superoxide dismutase (SOD1) are the primary cause of up to 20% of familial ALS cases.[14] Transgenic mice expressing human SOD1 mutations have been generated. These hSOD1 mutant transgenic mice exhibit pathological and cytological neuromuscular degeneration similar to patients with familial and some forms of sporadic ALS.[15–17] The hSOD1$^{G93A}$ mice are used for preclinical testing of compounds for treating ALS, since the disease in these animals follows a consistent onset, progression and outcome that mimics human ALS.[18–20] We previously showed that $\Delta^9$-THC was anti-excitotoxic in spinal cord cultures *in vitro*.[10] Here we report that $\Delta^9$-THC slows disease progression in hSOD1$^{G93A}$ mice when administered either before or after onset of signs. Furthermore, we demonstrate that $\Delta^9$-THC is extremely effective at reducing oxidative damage in spinal cord cultures, providing a cellular mechanism for its neuroprotective effect.

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## Materials and methods

### Transgenic mice

Male transgenic mice expressing the human SOD1$^{G93A}$ (B6SJL-TgN[SOD1-G93A]1Gur)( hSOD1$^{G93A}$ mice) were

bred with background matched B6SJL wild type females (Jackson Laboratories, Bar Harbor, ME). The total DNA was isolated from tail clips of the progeny by proteinase K digestion and subsequent phenol-chloroform extraction. The progeny were genotyped using primers specific to exon 4 of the human SOD1 gene within the transgenic construct and segregated and used for subsequent studies. Transgenic mice were housed in micro-isolator cages in a barrier facility and were seronegative for mouse hepatitis virus, Sendai virus and other common viral and bacterial pathogens. The mice were observed twice a week during the first 60 days of age and subsequently monitored every day for general health and signs of illness. Body weight was taken once a week and every day after the onset of disease.

### Treatment protocols

Male hSOD1$^{G93A}$ mice were injected intraperitoneally with vehicle (18:1:1 ratio of normal saline: emulphor: ethanol) or 5 or 10 mg/kg body weight $\Delta^9$-THC (Research Triangle Park, NIDA/NIH) dissolved in the same vehicle, daily from day 60 of their age. Alternatively, mice were administered 20 mg/kg $\Delta^9$-THC or vehicle beginning on day 75 when tremors were first observed. Mice were given *ad libitum* access to food and water, including moistened food on the floor after the onset of disease.

### Evaluation of motor function

Motor neuron function in mice was evaluated using a rotarod (Accuscan Instruments, Columbus, OH). Mice were trained on the rotarod for 10 minutes at speeds of 5 and 10 rpm beginning at 40 days of age. After the treatment started the mice were evaluated on the rotarod at 10 rpm on a weekly basis. The time they remained on the rotarod was registered automatically. If the mouse remained on the rod for 10 minutes, the test was completed and scored as 10 minutes. Mice were tested on the rotarod at 5 rpm after failing at 10 rpm in order to follow the decrement in motor function.

### Clinical end points

The clinical condition of mice was monitored twice a week after entry into each protocol and observed daily after entering into the treatment schedule. The earliest clinical signs observed were tremors and shaking of their limbs when mice were suspended briefly in the air by their tails. Progression of disease was measured by the decrement in the ability of mice to remain on the rotarod. To determine 'mortality' as an independent measure and humanely, mice were euthanized when they could not right themselves within 3 seconds after being placed on their sides.[20]

### Oxidative damage in mixed spinal cord cultures

Mixed spinal cord cultures were prepared from 13-day-old mouse embryos using methods previously described.[10,22] Experiments were performed after 1 week in culture. To induce oxidative damage, cultures were treated for 5 hours in the presence of 200 μM tert-butyl hydroperoxide (TBH).[23] The compounds evaluated were added at the same time as TBH. $\Delta^9$-THC (0.5 μM) and SR14716A (1 μM) were diluted from 1 mM stock solutions in ethanol; the final concentration of ethanol was kept constant in all treatments (0.1%). After the 5-hour treatment, cell viability was quantified by a combination of lactate dehydrogenase (LDH) activity and visually confirmed by propidium iodide fluorescence.[10]

### Statistical analysis

A logistic response curve was fitted to the endurance time for each mouse using a nonlinear mixed effects model.[24] The model is described by the equation:

$$\text{Time} = 10(1 + \exp((\text{Age} - A)/B))^{-1},$$

where Time is rod endurance time (range 0–10 mins), Age is mouse age in days, and A and B are constants representing day at which endurance is reduced to 50% (A) and rate of decline (1/B). The fitting program allows A and B to vary from mouse to mouse and can be used to test whether A and B differ by drug dose, delay in treatment and rpm of the rod. First we fitted the model allowing both A and B to vary from mouse to mouse, but neither A nor B were dependent on dose, delay or rpm. Next, we fitted a sequence of models starting with A and B each a linear function of dose and rpm, (A=A0+A1 Dose+A2 delay+A3 rpm, and B=B0+B1 Dose+B2 delay+B3 rpm). The coefficients for A and B and their standard errors were calculated by the program and Wald statistics were used to test whether each of the coefficients (A0, A1,...,B3) differed significantly from zero. A and B terms with nonsignificant ($P > 0.05$) coefficients were removed and the model was refitted. This process was repeated until all remaining terms were taken into account. Summary fit curves were prepared for all mice in each treatment group. Survival data were summarized by Kaplan-Meier curves.[25] For survival, a Cox proportional hazard model was fitted to the data and the effect of dose was tested by the likelihood ratio test and Wald statistics. First we tested for a difference among all 3 doses (log-rank test $P=0.003$). Then we tested pairwise differences finding that each dose effect on survival was significantly different from vehicle (0) dose ($P=0.004$ for 10 mg and $P=0.01$ for 20 mg) but that there was no difference between 10 and 20 ($P=0.56$). All calculations were carried out in S-Plus version 6. Mortality results are expressed as means of individual animals with s.e.m. per group. Results of *in vitro* experiments are expressed as means of cultures±s.e.m. per group. These data were assessed using an unpaired *t*-test with GraphPad Prism software (GraphPad, San Diego, CA).

## Results

### $\Delta^9$-THC delays disease progression and improves survival

The primary aim of this study was to evaluate the effectiveness of a cannabinoid in the treatment of a mouse model of ALS. A second aim was to improve the analysis of disease progression in the mouse model of ALS by using a more

powerful statistical model. hSOD1$^{G93A}$ mice were administered $\Delta^9$-THC (5 and 10 mg/kg body weight) or vehicle beginning at 60 days of age, i.e., prior to onset of motor dysfunction. The earliest clinical signs of disease observed were tremors and shaking of their limbs when mice were suspended briefly in the air by their tails.[18] These signs were never seen in non-transgenic littermates, but were always seen in hSOD$^{G93A}$ mice after 75 days. In a subsequent set of experiments, mice were administered 20 mg/kg $\Delta^9$-THC beginning on day 75 when tremors were first observed, i.e., after onset of disease signs. Mice were evaluated on a rotarod to follow disease progression. No changes in motor behavior were observed in the $\Delta^9$-THC treated animals at any of the doses given, confirming earlier reports.[21,26] Furthermore, no significant difference in weights was observed between the treatment groups.

In order to assess the effect of $\Delta^9$-THC on disease progression, a logistic response curve was fitted to the endurance time for each mouse using a nonlinear mixed effects model.[24] For these experiments, each mouse was assessed on the rotarod at 5 and 10 rpm.[27] Figure 1A shows the observed data and fitted endurance curves at 10 rpm for each mouse. The results from the nonlinear mixed effects model showed that both dose and rpm (but not delay in treatment initiation) significantly affected age at which endurance declined to 50% (i.e., 5 minutes, abbreviated as A50% henceforth). These results are summarized in Table 1 and shown graphically in Figure 1B. We found that A50% increased 3.3 days (±1.1 day) per 10 mg/kg of THC (2-sided $P$=0.003). In other words, disease progression as assessed by rotarod performance was delayed 3.3 days in the 10 mg/kg and 6.6 days in the 20 mg/kg group as compared to the vehicle treated animals. This represents a 3% increase in motor performance endurance in the 10 mg/kg group and a 6% increase in the 20 mg/kg group.

We were able to assess animals later in the disease by testing them at the slower rotarod speed (5 rpm). In the data analysis, we found that A50% increased 6.5 days (±0.4 day) when changing from 10 rpm to 5 rpm. However, compared with the rate of 10 rpm, the rate at 5 rpm was increased by a factor of 2. This rate of decline can also be inferred by expressing it as number of days to decline from 9 minutes to 1 minute endurance, based on the fitting equation, i.e., at 10 rpm, it took 17.1 (±1.76) days to decline from 9 to 1 minute compared with 8.55 (±0.88) days at 5 rpm. These results are summarized in Table 2 and shown graphically in Figure 1B.

In these experiments, the treatment effect was to slow the progression of disease (Figure 1b). Furthermore, treatment with $\Delta^9$-THC improved survival. Treatment with 10 mg/kg $\Delta^9$-THC extended mean survival from 125.9±1.6 days (vehicle, $n$=15) to 131.8±2.4 days ($\Delta^9$-THC, $n$=8, $P$=0.004) (Figure 2a, c). This represents a 4.9 day (4.6%) increase in survival in the 10 mg/kg $\Delta^9$-THC-treated group. At a (delayed) dose of 20 mg/kg the increase in mean survival was 6.4 days (5.1%) ($P$=0.01, Figure 2a,c).

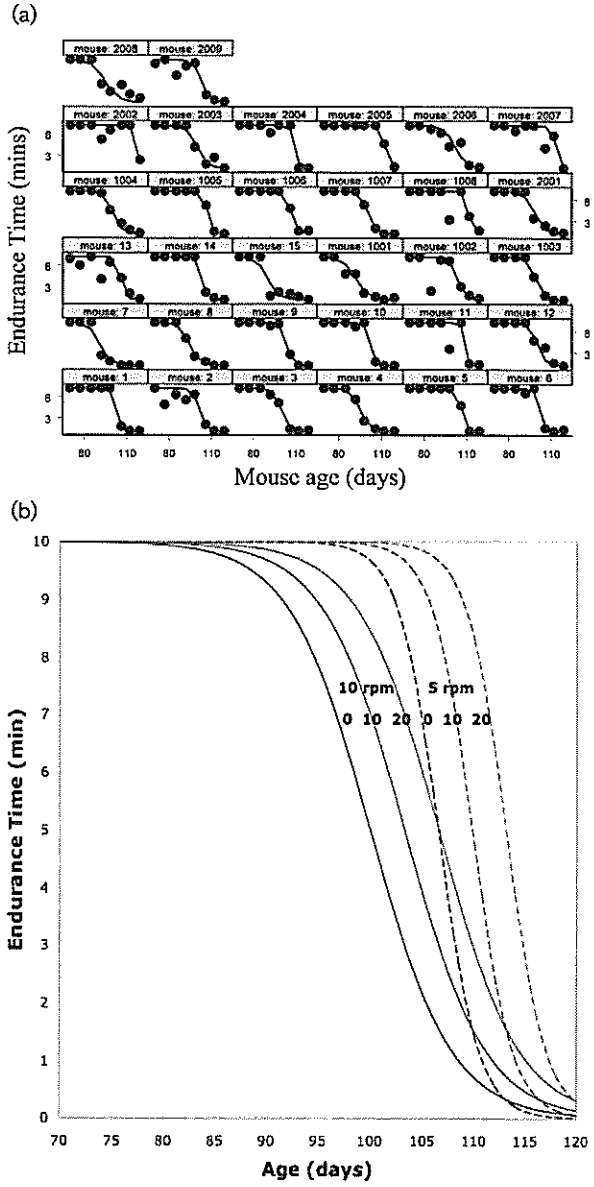



**Figure 1**
$\Delta^9$-THC delays progression of disease in hSOD1$^{G93A}$ mice. Motor function was tested weekly on the rotarod at 10 and 5 rpm. The decline in endurance over time for each animal at 10 rpm is shown in (**a**). Mouse numbers 1–13 correspond to vehicle treated animals, numbers 1001–1008 correspond to the 10 mg/kg $\Delta^9$-THC treatment group, and 2001–2009 correspond to the 20 mg/kg $\Delta^9$-THC treatment group. In (**b**) the curves show declines based on fitting a logistic model to observed data. Solid curves are tests at 10 rpm, dashed curves for 5 rpm. The three curves for each rpm are for doses of 0 (vehicle, blue), 10 (mg/kg $\Delta^9$-THC, green) and 20 (mg/kg $\Delta^9$-THC, red). Parameters for curves are based on a nonlinear mixed effects model given by Time=10/(1+exp((Age−A)/B)).

## $\Delta^9$-THC is anti-oxidant and anti-excitotoxic *in vitro*

$\Delta^9$-THC is known to be effective in attenuating *in vitro* excitotoxic and oxidative cell damage. Both of these mechanisms have been implicated in the progression of ALS. We had previously reported that $\Delta^9$-THC was as effective as the anti-excitotoxic compound NBQX, an AMPA/Kainate receptor antagonist, in protecting spinal cord neurons against direct excitotoxin (kainate) exposure.[10] To evaluate the possibility that $\Delta^9$-THC may also have antioxidant properties in spinal

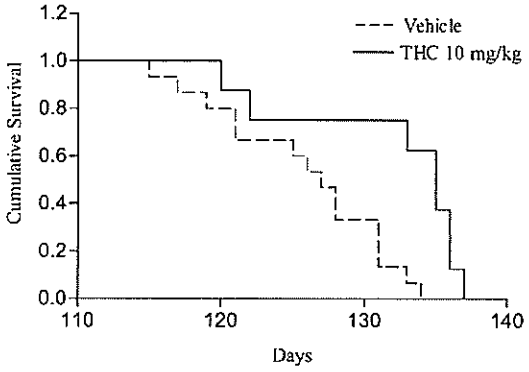
| Factor | Estimate | Std Error | DF | *t*-statistic | *P*-value |
|---|---|---|---|---|---|
| A.(intercept) | 109.78 | 2.31 | 477 | 47.44 | <.0001 |
| A.(dose) | 3.29 | 1.12 | 477 | 2.95 | 0.0034 |
| A.(rpm) | −6.54 | 0.44 | 477 | −14.90 | <.0001 |
| B.(rpm) | 1.95 | 0.20 | 477 | 9.67 | <.0001 |

**Table 1**
Summary of results

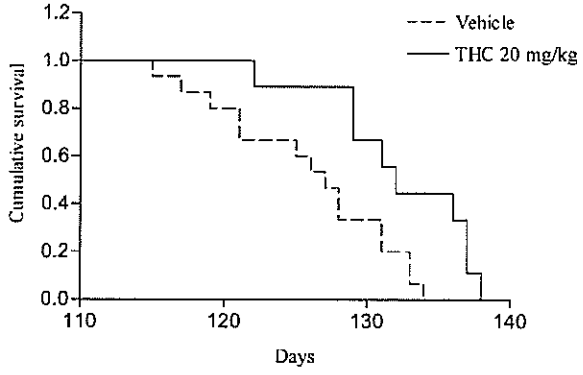
| rpm | Dose | Days to 50% endurance | Days from 9 to 1 min |
|---|---|---|---|
| 5 | 0 | 106.5 | 8.55 |
| 5 | 10 | 109.8 | 8.55 |
| 5 | 20 | 113.1 | 8.55 |
| 10 | 0 | 100.0 | 17.10 |
| 10 | 10 | 103.3 | 17.10 |
| 10 | 20 | 106.6 | 17.10 |

**Table 2**
Predictions based on model

**a**




**b**




**c**

| | Vehicle ($n$=15) | $\Delta^9$-THC 5 mg/kg ($n$=7) | $\Delta^9$-THC 10 mg/kg ($n$=8) | $\Delta^9$-THC 20 mg/kg ($n$=9) |
|---|---|---|---|---|
| Mortality (days) | 125.9 ± 1.6 | 128.4 ± 4.3 (NS) | 131.8 ± 2.4 * | 132.3 ± 1.7 * |

**Figure 2**
$\Delta^9$-THC extends survival in hSOD1$^{G93A}$ mice. Cumulative survival in hSOD1$^{G93A}$ mice treated with 10 mg/kg $\Delta^9$-THC, begun at 60 days of age (**a**) or 20 mg/kg $\Delta^9$-THC, started at 75 days of age. Vehicle controls were treated at 60 days and 75 days of age, respectively; no difference was seen in the lifespan between the two vehicle treatment paradigms, so the data were pooled for analysis. (**b**). Mortality of hSOD1$^{G93A}$ mice treated with $\Delta^9$-THC or vehicle (**c**). Survival was significantly increased in the 10 and 20 mg/kg $\Delta^9$-THC treated groups compared to vehicle controls (*, $P$=0.004, $P$=0.01, respectively, error bars represent s.e.m.).

cord cultures, we determined whether $\Delta^9$-THC could protect against oxidative damage produced by direct application of the oxidant tert-butyl hydroperoxide (TBH).[23] $\Delta^9$-THC was extremely effective at reducing oxidative damage produced by TBH in mixed spinal cord cultures. Exposure to 200 $\mu$M TBH for 5 hours resulted in 74 ($\pm$14) % cytotoxicity, which was reduced to 28 ($\pm$8) % in the presence of 0.5 $\mu$M $\Delta^9$-THC ($n$=4, $P$<0.001)(Figure 3). These quantitative data were visually confirmed by measuring propidium iodide uptake in parallel cultures (data not shown); both neurons and glia were affected by TBH as previously published.[23] To test if this profound antioxidant effect was $CB_1$ receptor mediated, 1 $\mu$M SR141716A was used to block the receptor (Figure 3). There was no difference in the level of protection, suggesting the antioxidant effect was not $CB_1$ receptor mediated.

## Discussion

Our study is innovative in at least two characteristics. First, treatment at 20 mg/kg began after first appearance of disease signs. This design more realistically mimics treatment in humans where it is not possible to treat prior to symptom onset. Secondly, the use of a nonlinear mixed effects model for data analysis increased statistical power to detect a large range of effects and to test separately for delayed disease onset and rate of disease progression. Use of the model eliminates the complication of defining an age at onset based on observed symptoms. The use of a logistic model provides an estimate of the age at which muscle endurance has declined by 50% with much greater accuracy than could be attained for any other measure of decline. This is because statistically maximum precision is attained at the median of a logistic regression. Use of the logistic model also allowed us to test whether the dose effect at 20 mg/kg was diminished by delaying treatment until the first appearance of disease signs. The results showed that endurance and survival could be increase in the $hSOD^{G93A}$ mice even when $\Delta^9$-THC was administered after the onset of disease.

The mechanisms for neuroprotection by $\Delta^9$-THC appear to

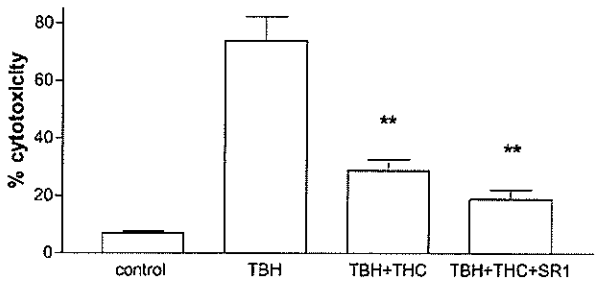



**Figure 3**

$\Delta^9$-THC attenuates oxidative stress in mouse spinal cord cultures. Mouse primary spinal cord cultures were exposed to the oxidant tert-butyl hydroxide in vehicle (TBH), or in the presence of 0.5 $\mu$M $\Delta^9$-THC (TBH + THC) or 0.5 $\mu$M $\Delta^9$-THC plus 1 $\mu$M SR141716A (TBH + THC + SR1). $\Delta^9$-THC was extremely effective at reducing TBH cytotoxicity as assessed by LDH release; this effect was not reversed by the $CB_1$ receptor antagonist SR141716A (**, $P$<0.001, $n$=3–6, error bars represent s.e.m.).

be multifaceted. In this study, the antioxidant neuroprotective effect of $\Delta^9$-THC was not $CB_1$-receptor mediated because the $CB_1$ receptor antagonist SR141716A did not diminish the antioxidant effect. $CB_1$ receptors were detected in neurons and glia in the spinal cord cultures by immunocytochemistry and Western blot analysis, thus the lack of effect of receptor antagonist is not due to the absence of receptors in the cultures.[10] $CB_2$ receptor expression has not been demonstrated in neurons or astrocytes though we can readily detect $CB_2$ receptor expression in cells from the immune system.[28] Other cannabinoid receptor subtypes have been proposed, however they have not been identified. Thus while it is possible that other cannabinoid-type receptors could be involved with the antioxidant effect, there are no reagents to test this possibility. Previous investigations into the potential neuroprotective effects of cannabinoids have focused on models of cerebral ischemia[9,29], multiple sclerosis[11] and epilepsy.[26] Our results are in line with the *in vitro* models of cerebral ischemia in which the predominant neuroprotective effect of cannabinoids is as an antioxidant but not $CB_1$ receptor mediated.[7,29] In addition, cannabinoids were equally effective as neuroprotective antioxidants in cultured neurons from $CB_1$ receptor knockout mice or control wild-type littermates.[30] Furthermore, cannabidiol and (+)11-OH-$\Delta^8$-THC (HU-211) which have no $CB_1$ receptor activity are also effective antioxidants.[7] $\Delta^9$-THC, cannabidiol and HU-211 were found to possess greater antioxidant properties than either ascorbate or $\alpha$-tocopherol, similar to that of BHT.[7]

The antioxidant properties of $\Delta^9$-THC may also contribute to the attenuation of excitotoxicity in primary neuronal cultures.[7,31] We previously found that $\Delta^9$-THC protected against kainate-mediated toxicity in mixed (neuronal and glial) spinal cord cultures.[10] The amount of cytotoxicity produced by kainate was about half of that produced by the oxidative stress paradigm described in this report, but is consistent with a neuronal toxicity (excitotoxicity) as opposed to a combined neural and glial toxicity (oxidative stress).[23] That $\Delta^9$-THC protects glia as well as neurons may contribute towards its effectiveness in the $hSOD^{G93A}$ mice. Astrocytic cell damage is believed to contribute to the disease process by suppressing the activity of EAAT2 glutamate transporters that are necessary for recovering synaptic glutamate and/or preventing repetitive motor neuron firing.[3]

A potential therapeutic application of $\Delta^9$-THC for the symptoms of ALS includes the relief of spasticity.[6,32–34] Similar results were found in a mouse model of multiple sclerosis in which cannabinoids quantitatively ameliorate both tremor and spasticity.[11] Recently, increased levels of the endogenous ligands anandamide, 2-arachidonylglycerol and palmitoylethanolamide were found in the brain and spinal cord, areas associated with the induced nerve damage.[12] The endogenous ligands were also anti-spastic; in addition, inhibitors of re-uptake and hydrolysis were shown to significantly attenuate spasticity.[12]

In order for a pharmacological treatment to be clinically effective for ALS, it must be able to protect the remaining motor neurons (as up to 50% are lost at the time of clinical diagnosis).[35] Other treatments to date have focused on antioxidants, neurotrophic, immunosuppressive and neuroprotective

agents, most of which have been shown to be neuroprotective in cellular and animal models of ALS.[35] Nevertheless, over 25 large clinical studies based on these strategies have shown no benefit, apart from riluzole.[35,36] As a result, riluzole is the only drug approved and marketed for the treatment of ALS.[37] However, candidate compounds are usually tested in the mouse model by administration prior to the onset of disease signs, which may explain their failure in clinical trials.

An important additional consideration is that ALS is a chronic disease, therefore long-term toxicity of treatment drugs becomes an important issue. $\Delta^9$-THC is well tolerated and already in clinical usage for nausea associated with cancer chemotherapy and appetite stimulation with the AIDS wasting syndrome. In a pilot study of the safety and tolerability of $\Delta^9$-THC in ALS patients, symptomatic benefits were seen in insomnia, appetite and spasticity.[13] $\Delta^9$-THC is effective in a pre-clinical model of ALS, suggesting it could be evaluated for its effectiveness in the human disease.

## Conclusion

The data presented here indicate that $\Delta^9$-THC delays progression of disease and increases survival time in $hSOD^{G93A}$ mice even when administered after onset of signs. Our finding that $\Delta^9$-THC is both anti-excitotoxic and antioxidant *in vitro* suggests it may act additively towards its therapeutic effect in the $hSOD^{G93A}$ mice.

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# REFERENCE #2

# The Endocannabinoid System in Amyotrophic Lateral Sclerosis

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**Abstract:** Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative condition characterised by the selective loss of motor neurons from the spinal cord, brainstem and motor cortex. Although the pathogenic mechanisms that underlie ALS are not yet fully understood, there is significant evidence that several neurotoxic mechanisms including excitotoxicity, inflammation and oxidative stress, all contribute to disease pathogenesis. Furthermore, recent results have established that although primarily a motor neuron specific disorder, ALS is not cell-autonomous and non-neuronal cells including astroglia and microglia play a critical role in mechanism of disease. Currently the only licensed therapy available for the treatment of ALS is the anti-glutamatergic agent Riluzole, which has limited therapeutic effects. However, there is increasing evidence that cannabinoids and manipulation of the endocannabinoid system may have therapeutic value in ALS, in addition to other neurodegenerative conditions. Cannabinoids exert anti-glutamatergic and anti-inflammatory actions through activation of the $CB_1$ and $CB_2$ receptors, respectively. Activation of $CB_1$ receptors may therefore inhibit glutamate release from presynaptic nerve terminals and reduce the postsynaptic calcium influx in response to glutamate receptor stimulation. Meanwhile, $CB_2$ receptors may influence inflammation, whereby receptor activation reduces microglial activation, resulting in a decrease in microglial secretion of neurotoxic mediators. Finally, cannabinoid agents may also exert anti-oxidant actions by a receptor-independent mechanism. Therefore the ability of cannabinoids to target multiple neurotoxic pathways in different cell populations may increase their therapeutic potential in the treatment of ALS. Recent studies investigating this potential in models of ALS, in particular those that focus on strategies that activate $CB_2$ receptors, are discussed in this review.

**Key Words:** Neurodegeneration, excitotoxicity, inflammation, oxidative stress, SOD1, neuroprotection, motor neuron, therapy.

## INTRODUCTION

Originally described in 1869 by the French physician Jean-Martin Charcot, Amyotrophic Lateral Sclerosis (ALS) is the most common adult motor neuron disease in humans. ALS is characterised by the progressive degeneration of lower motor neurons in the spinal cord and brainstem, and the large pyramidal neurons in the motor cortex and associated corticospinal tracts. ALS is predominantly a sporadic disorder, although approximately 10% of cases have a familial history consistent with Mendelian inheritance. Following the discovery that a subset (approximately 20%) of familial ALS is caused by mutations in the Cu/Zn superoxide dismutase (SOD1) enzyme [1], transgenic mice expressing various mutant human SOD1 genes have been generated and have been widely used as a model for the disease. These transgenic mice develop a motor neuron disease with many of the pathological features seen in humans including neurofilament inclusions, ubiquitinylated aggregates and selective motor neuron loss, accompanied by severe muscle paralysis and premature death. These mice therefore providing a valuable research tool for the study of ALS pathogenesis and preclinical testing of potential therapies (reviewed in [2]).

Extensive research using SOD1 mice, in combination with analysis of post-mortem tissue from ALS patients has provided insight into the neurotoxic mechanisms that are involved in ALS. There is now strong evidence that implicates glutamate excitotoxicity, inflammation and oxidative stress, amongst other mechanisms, in ALS pathogenesis (reviewed in [2]). In this Review, we will discuss the evidence for the involvement of these mechanisms in ALS pathogenesis and then summarise the actions of the endocannabinoid system in targeting these mechanisms (see Fig. **1**), in order to assess the therapeutic potential of modulation of the endocannabinoid system as a strategy for the treatment of ALS. A summary of the various studies in which the endocannabinoid system has been manipulated in models of ALS is shown in Table **1**.

## TARGETING OF GLUTAMATE EXCITOTOXICITY BY THE ENDOCANNABINOID SYSTEM IN ALS

Glutamate is the most abundant excitatory neurotransmitter in the CNS [3]. However, there is substantial evidence to suggest that over-activation of glutamate receptors can result in neuronal damage, a process termed 'excitotoxicity'. Elevated extracellular glutamate, either due to an increase in release or a reduction in uptake, can activate glutamate receptors on the postsynaptic cell, thus enhancing calcium influx. Excessive postsynaptic calcium can subsequently activate neurotoxic cascades such as activation of calpains, endonucleases and phospholipases, ultimately leading to neuronal death [4].

Motor neurons receive glutamatergic inputs from the descending corticospinal tracts, from collaterals of the Aα fibres innervating muscle fibres and Golgi tendon organs and

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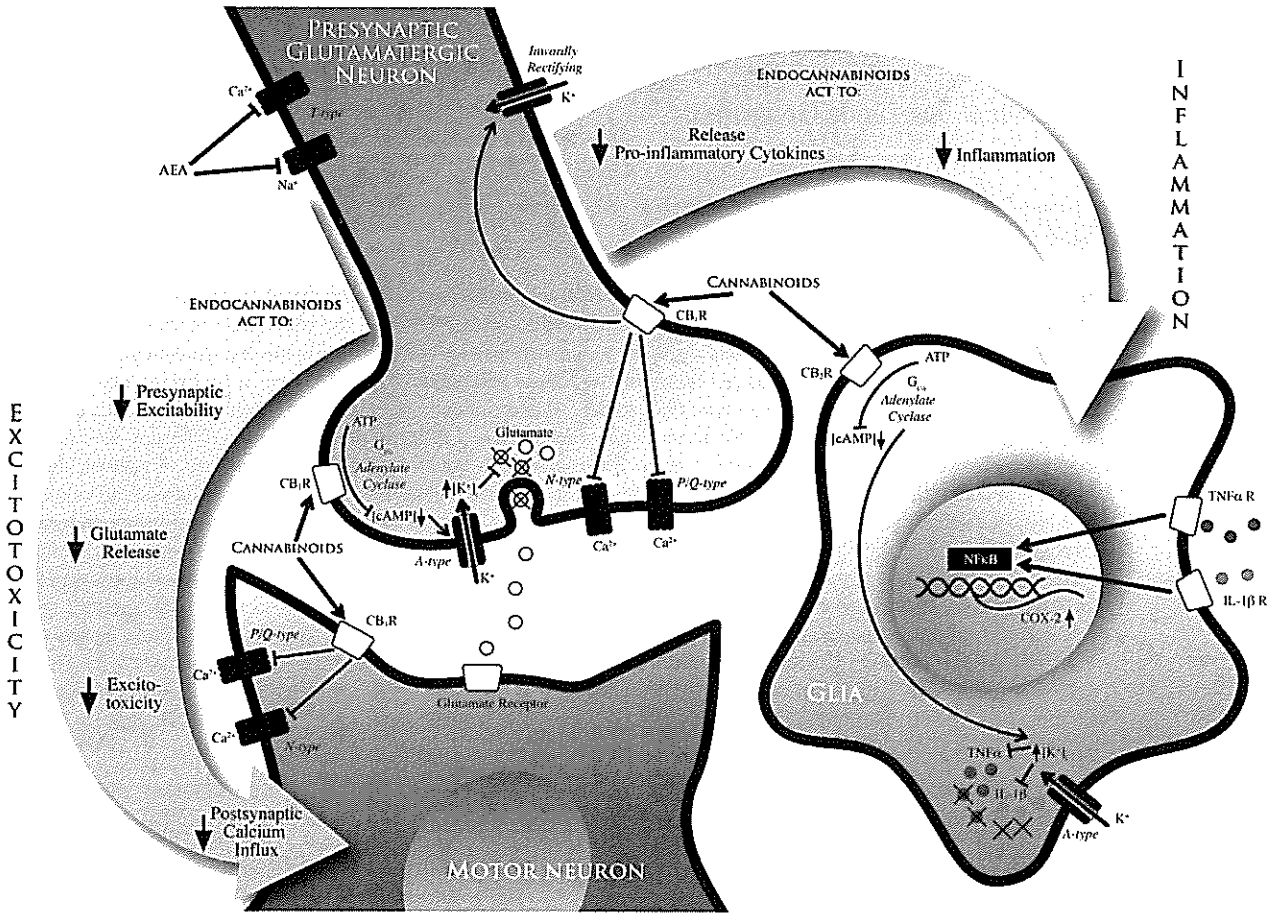


**Fig. (1). Summary of the potential neuroprotective mechanisms of the endocannabinoid system in ALS.**

Endocannabinoids may exert neuroprotective actions in ALS by targeting two main neurotoxic mechanisms; excitotoxicity and inflammation. This illustration gives a basic overview of the potential neuroprotective actions of endocannabinoids in ALS, and shows the actions of endocannabinoids against excitotoxicity on the left hand side and against inflammation on the right hand side. Activation of $CB_1$ receptors by cannabinoids may alter the calcium and potassium permeability of the pre- and postsynaptic neuron reducing their excitability. This will result in the inhibition of glutamate release, causing a reduction in postsynaptic calcium influx, thereby minimising the effects of excitotoxicity. Meanwhile $CB_2$ receptor activation will similarly act to reduce cellular excitability and the localisation of the $CB_2$ receptor to glial cells will result in a reduction in the release of proinflammatory cytokines such as TNFα and IL-1β, thereby inhibiting further propagation of the inflammatory response, so that for example COX-2 levels do not increase. The endocannabinoid system may also exert neuroprotective effects in a non-receptor mediated manner, although these mechanisms are not depicted in this diagram.

**Abbreviations:** $K^+$: potassium; $Ca^{2+}$: calcium; $CB_1$: cannabinoid receptor 1; $CB_2$: cannabinoid receptor 2; cAMP: cyclic AMP.

from excitatory interneurons in the spinal cord [3]. Therefore, abnormalities in glutamate neurotransmission may have a particularly significant impact on motor neurons and may thereby contribute to motor neuron degeneration in ALS. Indeed, an elevation in cerebrospinal fluid glutamate levels occurs in approximately 40% of sporadic ALS patients [5, 6] and transcranial magnetic stimulation studies in ALS patients reveal that cortical motor neurons are hyperexcitable [7]. Furthermore, it has been reported that there is an increase in firing frequency and a reduction in action potential duration in embryonic motor neurons *in vitro,* which further indicates that there may be an increase in motor neuron excitability in ALS [8, 9].

In addition to an increase in motor neuron excitability, a reduction in glutamate uptake may also contribute to excitotoxicity in ALS. Reduced expression of the main excitatory amino acid transporter-2 (EAAT-2, also known in mice as glutamate transporter-1, GLT-1) and a decrease in glutamate transport has been found in 60-70% of sporadic ALS postmortem spinal cord and motor cortex tissue [10, 11]. A mutation in EAAT-2 has also been identified in a sporadic ALS patient and was associated with a reduction in glutamate uptake [12]. Similarly, glutamate uptake is diminished in symptomatic SOD1 mice, and this is reflected in a reduction in EAAT2 (GLT-1) expression [13-15]. Recently, mutant SOD1 has been shown to have the ability to induce caspase-3 mediated cleavage of EAAT-2 (GLT-1) [16]. Indeed, an inactive, truncated form of EAAT2 (GLT-1) is found in spinal cords of presymptomatic SOD1 mice [16]. This fragment is sumoylated and accumulates in promyelocytic leukaemia

**Table 1. Summary of Studies Manipulating the Endocannabinoid System in *In Vitro* and *In Vivo* Models of ALS**

| Manipulation of Endocannabinoid System | Dose and Onset of Treatment | Potential Mechanism of Action | Effects in ALS Models | Ref. |
|---|---|---|---|---|
| WIN-55,212-2 | *In vivo:* 5mg/kg from symptom onset | Activation of $CB_1$ and $CB_2$ receptors. | Delayed disease progression in SOD1 mice<br>4% increase in lifespan of SOD1 mice | [87]<br>[84] |
| AM-1241 | *In vivo*: 3mg/kg<br>0.3mg/kg from symptom onset | Selective $CB_2$ receptor agonist | Extended lifespan of SOD1 mice by 11%<br>Extended lifespan of SOD1 mice by 7% | [84] |
| AM-1241 | *In vivo*: 1mg/kg presymptomatically | Selective $CB_2$ receptor agonist | Delayed disease progression in SOD1 mice | [38] |
| $\Delta^9$-THC | *In vivo*: 20mg/kg from symptom onset | Activation of $CB_1$ and $CB_2$ receptors, also receptor independent anti-oxidative actions | Delayed disease progression in SOD1 mice and extended lifespan by 5% | [85] |
| $\Delta^9$-THC | *In vitro:* 0.5μM | Activation of $CB_1$ and $CB_2$ receptors, also receptor independent anti-oxidative actions | Neuroprotective against kainate mediated toxicity in motor neurons *in vitro* | [53] |
| Cannabinol | *In vivo*: 5mg/kg presymptomatically | Non-psychoactive cannabinoid. Binds to $CB_1$ and $CB_2$ receptors but with lower affinity than $\Delta^9$-THC | Delayed disease onset in SOD1 mice | [145] |
| *Faah* ablation | *In vivo*: From conception | Elevated AEA levels | Delayed disease progression in SOD1 mice | [87] |
| $CB_1$ receptor ablation | *In vivo* : From conception | Ablates potential neuroprotective contribution of $CB_1$ receptors | Extended lifespan in SOD1 mice by 13% | [87] |

Several manipulations of the endocannabinoid system have been tested in models of ALS, with varying results. This table summarises the results of these studies to date.

nuclear bodies, which may interfere with their normal regulatory role in gene transcription [17]. *In vitro*, in organotypic spinal cord cultures, chronic inhibition of glutamate transport induces selective motor neuron degeneration [18], whereas restoration of EAAT2 (GLT-1) activity either by pharmacological enhancement [19] or by the introduction of glial progenitor cells over-expressing EAAT2 (GLT-1), significantly increases motor neuron survival [20].

Glutamate-mediated excitotoxicity in ALS is thought to result from activation of calcium permeable AMPA/KA receptors [18, 21-23]. Indeed, activation of AMPA/KA receptors induces selective degeneration of motor neurons *in vitro* [18, 24, 25] and in mice *in vivo* [26]. This effect is inhibited by selective AMPA/KA receptor antagonists *in vitro* [21-23, 27] and treatment of SOD1 mice with AMPA/KA receptor antagonists *in vivo,* significantly extends their lifespan [27-29].

AMPA receptors mediate fast neurotransmission and are composed of 4 different subunits, GluR1-4, which confer different functional characteristics [30]. The absence of the GluR2 subunit renders the AMPA receptor calcium permeable [30]. Expression of GluR2 in motor neurons is regulated by surrounding astrocytes, which therefore play an important role in determining the vulnerability of motor neurons to excitotoxicity [31]. A large number of studies have shown that motor neurons express GluR2-containing AMPA receptors [23, 32-35], although there is some evidence for the co-localisation of GluR2-containing and GluR2-lacking AMPA receptors on the same motor neurons [36]. However, defective GluR2 editing, which would render the receptor calcium permeable, has been found selectively in motor neurons in post-mortem spinal cord tissue from ALS patients [37]. Furthermore, a specific reduction in GluR2 expression was recently identified in motor neurons from presymptomatic SOD1 mice, which may render them more susceptible to excitotoxicity [29, 38].

It is likely that the selective vulnerability of motor neurons to excitotoxicity may be related to a higher density of calcium-permeable AMPA receptors, which will result in an increase in agonist-induced calcium influx into these neurons [23, 24, 27, 39]. Reducing the calcium permeability of AMPA/KA receptors by crossing SOD1 mice with mice over-expressing the GluR2 subunit, significantly delays symptom onset and extends their lifespan [40]. In contrast, acceleration in disease course and a shortening of lifespan is seen in SOD1 mice following manipulations that result in an increase in calcium permeability or the total ablation of the GluR2 subunit [41, 42]. However, there is no motor neuron loss in GluR2 knock-out mice, suggesting that either the absence of the GluR2 subunit alone is not sufficient to induce ALS [43] or that compensatory mechanisms can ameliorate the effects of the lack of GluR2 expression in motor neurons.

The vulnerability of specific populations of motor neurons to glutamate-induced excitoxicity is further exacerbated by a reduced ability to bind calcium. Thus, specific motor neuron populations that appear to be selectively vulnerable to degeneration in ALS, do not express the calcium binding proteins calbindin-D(28k) and parvalbumin, whereas disease-resistant motor neurons, such as occulomotor neurons and motor neurons in the Onuf's nucleus [44, 45] express high levels of these calcium binding proteins [46, 47]. Motor neurons with a low expression of calcium binding proteins

may therefore have to depend to a greater extent on mitochondrial calcium uptake in order to buffer excess calcium, and this may render their mitochondria more susceptible to damage, a common feature in ALS. In support of the proposal that a decrease in calcium buffering capacity contributes to the selective vulnerability of motor neurons in ALS, it has been found that over-expression of parvalbumin protects motor neurons *in vitro* against KA-induced calcium influx [48], and protects neonatal motor neurons from injury-induced motor neuron death in parvalbumin over-expressing transgenic mice [49]. Furthermore, the survival of SOD1 mice crossed with parvalbumin over-expressing transgenic mice is also extended [50].

Together, these findings highlight excitotoxicity as a significant pathogenic mechanism in motor neuron degeneration in ALS and, as discussed above, strategies that reduce the effects of excitotoxicity by antagonising AMPA/KA receptors or by elevating the expression of EAAT-2 (GLT-1) or calcium binding proteins, have beneficial effects in models of ALS. However, despite the fact that cannabinoids have been shown to be neuroprotective in a number of experimental models of excitotoxicity [51-59], until recently, few studies have examined the possible beneficial effects of cannabinoids in ALS. Indeed, due to the activity-dependent nature of their synthesis, it is possible that endocannabinoid synthesis represents an endogenous defence mechanism that is upregulated under excitotoxic conditions [60, 61]. Following injection of excitotoxins into the CNS of mice, levels of the endocannabinoids, N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) significantly increase [55, 62-64]. This effect may be mediated by activation of postsynaptic group I metabotropic glutamate receptors (mGluR), which will elevate intracellular calcium levels and activate phospholipase C, subsequently inducing endocannabinoid formation (reviewed in [65]). Elevations in endocannabinoids in response to excitotoxicity suggest an adaptive role for endocannabinoids in protection against excitotoxicity. These anti-excitotoxic actions represent a very significant mechanism by which the endocannabinoid system may have therapeutic value in ALS.

Cannabinoid-mediated neuroprotection against excitotoxicity is predominantly the result of activation of $CB_1$ receptors [66] (see Fig. **1**) $CB_1$ receptors are coupled to inhibitory GTP binding proteins $G_{i/o}$. Thus their activation can inhibit adenylate cyclase. The resultant reduction in cyclic AMP levels and subsequent inhibition of protein phosphorylation, may inhibit neuronal activity *via* activation of A-type potassium channels [67]. Furthermore, activation of $CB_1$ receptors mediates inhibition of voltage gated N- and P-/Q- type calcium channels [68-71] and activation of inwardly rectifying potassium channels [70], independently of adenylate cyclase inhibition. This presynaptic regulation of calcium and potassium permeability will raise the threshold for neurotransmitter release, thereby decreasing overall release. Indeed *in vitro,* cannabinoids have been shown to inhibit the release of neurotransmitters [72-74]. In addition, activation of postsynaptic $CB_1$ receptors may also reduce excitotoxicity-induced postsynaptic calcium influx *via* inhibition of voltage gated N- and P/Q- type calcium channels [53, 68-71, 75]. Therefore under conditions of excitotoxicity, activation of $CB_1$ receptors may reduce both presynaptic glutamate release and the excitability of the postsynaptic cell. Furthermore, the endogenous ligand AEA and the synthetic cannabinoid receptor agonist WIN55,212-2, have been shown to directly interact with and inhibit, sodium channels and T-type calcium channels, actions which would also reduce neuronal excitability and further hinder action potential propagation [74, 76].

Several other signalling pathways may also contribute to the effects of $CB_1$ receptor activation. Activation of the $CB_1$ receptor leads to induction of mRNA expression of immediate early genes, such as *c-fos*, involved in neuroprotection against excitotoxicity [55], and *Krox 24*, a growth related gene [77], an effect that is mediated by $G_i$ protein-dependent activation of extracellular signal regulated kinase [55] and mitogen activated protein kinase respectively [77].

Thus, $CB_1$ receptor activation can potentially inhibit the release and postsynaptic effects of excitotoxic levels of glutamate, therefore providing protection against excitotoxicity. In support of this, it has been shown that injection of kainate (KA) into the central nervous system (CNS) of $CB_1$ receptor knock-out mice produces a more extreme behavioural reaction and a greater mortality than in wild-type (WT) mice [55, 57]. More specifically, Marsicano *et al.,* (2003) generated a conditional knock–out mouse model in which only $CB_1$ receptors on glutamatergic neurons of the forebrain were deleted. Administration of KA to these mice induces seizures that are significantly worse than in mice with a full complement of $CB_1$ receptors. These results imply that $CB_1$ receptors, particularly those located on glutamatergic neurons, play a role in endogenous protection against excitotoxicity and represent a system that may be suitable for therapeutic manipulation by administration of exogenous cannabinoid agonists.

The $CB_1$ receptor is the most predominant cannabinoid receptor subtype in the CNS [78] with the highest densities found in areas including the basal ganglia, the molecular layers of the cerebellum and in portions of the hippocampus [79]. In cultured motor neurons, $CB_1$ receptors are expressed on the cell soma and neurites [53], although the levels of expression in the ventral horn of the spinal cord are relatively low compared to the basal ganglia [79]. Cultured astrocytes and microglial have also been shown to express $CB_1$ receptors [80-82], suggesting that during disease progression, overall $CB_1$ receptor density in the spinal cord may increase due to the influx of microglia to areas affected in ALS (reviewed in [83]). Indeed, $CB_1$ receptor mRNA expression is significantly increased at a symptomatic stage of disease in SOD1 mice, although this is not maintained at disease end-stage [38, 84].

In view of the significant role that excitotoxicity plays in ALS pathogenesis together with the ability of the endocannabinoid system to modulate excitotoxic mechanisms, it has been suggested that cannabinoids may have significant neuroprotective effects in ALS. Certainly, there is now significant evidence to suggest that in models of ALS, cannabinoids can be neuroprotective through anti-excitotoxic mechanisms. Treatment with $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) protects mouse spinal cord neurons *in vitro* against direct KA-induced toxicity, an effect blocked by SR141716A, a selective $CB_1$ receptor antagonist [53]. Furthermore, treat-

ment of SOD1 mice with $\Delta^9$-THC *in vivo,* delays disease progression [85] (see Table **1**). Interestingly, in the spinal cord of SOD1 mice, levels of AEA and 2-AG increase from symptom onset [86, 87]. This increase may be due, at least in part, to an up-regulation in expression of functional mGluR5 on SOD1 astrocytes compared to normal astrocytes, which on activation would lead to significant elevations in endocannabinoid levels [65, 88]. This is likely to be an adaptive response aimed at counteracting excitotoxicity, similar to that observed in the hippocampus of mice treated with KA [55].

Cannabinoids may also exert anti-excitotoxic effects in a non-$CB_1$ receptor dependent manner. In the brain of $CB_1$ receptor knock-out mice, an as yet unidentified, G-protein coupled cannabinoid "$CB_3$" receptor has been pharmacologically characterised [89, 90]. This receptor is activated to the same extent by AEA and synthetic cannabinoid WIN55,212-2, but not by other synthetic cannabinoids or $\Delta^9$-THC. It is insensitive to $CB_1$ and $CB_2$ receptor antagonists and is not coupled to the inhibition of adenylate cyclase [90], unlike the existing cannabinoid receptors. There is, however, evidence to suggest that this receptor may be selectively coupled to the inhibition of glutamate release in the mouse hippocampus [91]. Further investigation is required to confirm whether activation of this receptor may also have beneficial effects under excitotoxic conditions in models of ALS.

However, despite the established anti-excitotoxic effects of endocannabinoids, there is also evidence that suggests that the endocannabinoid system may be capable of exacerbating excitotoxicity. For example, in hippocampal neurons, endocannabinoids are released in response to depolarisation and activate $CB_1$ receptors on neighbouring astrocytes. In this case, $CB_1$ receptors coupled to $G_s$ proteins elevate cyclic AMP levels causing an increase in intracellular calcium levels, thereby stimulating the release of glutamate [92, 93]. This glutamate can subsequent activate N-methyl D-aspartate (NMDA) receptors on neighbouring pyramidal neurons [94]. These results show that potentially hyperexcitable motor neurons can release elevated levels of endocannabinoids [86, 87], which under conditions where $G_{i/o}$ protein signal transduction pathways are inhibited, may ultimately contribute to neuronal degeneration, albeit indirectly. This finding may explain the general inability of cannabinoids, which have an element of $CB_1$ receptor activation in their mechanism of action, to extend the lifespan of SOD1 mice despite initially displaying a neuroprotective effect [85, 87].

## TARGETING OF INFLAMMATION BY THE ENDOCANNABINOID SYSTEM IN ALS

Over recent years, increasing evidence has highlighted the significant contribution that inflammation plays in ALS pathogenesis. Examination of post-mortem spinal cord tissue from ALS patients shows that there are substantial signs of inflammation including significant proliferation and accumulation of activated microglia, reactive astrocytes and $CD4^+$ and $CD8^+$ lymphocytes in areas of motor neuron degeneration (reviewed in [83]). Furthermore, increases in mRNA and protein levels of inflammatory markers such as COX2 and $PGE_2$ are also seen in post-mortem spinal cord tissue [95, 96]. Similar neuroinflammatory changes are also seen in SOD1 mice and these appear to correlate with disease progression, so that for example reactive astrogliosis increases steadily from symptom onset. However, microglial activation is evident from a presymptomatic age and continues to increase in intensity throughout disease progression, paralleling the loss of motor neurons [97-99]. In fact, increased expression of intracellular adhesion molecule-1 (ICAM-1) and NADPH oxidase, the main reactive oxygen species (ROS) producing enzyme in inflammation, on microglia are some of the earliest pathological changes seen in the SOD1 mice, and this up–regulation may be important in the induction of inflammatory processes [98, 100, 101].

Activated microglia and astrocytes release a variety of neurotoxic mediators including pro-inflammatory cytokines, such as tumour necrosis factor α (TNFα) and interleukin-1β (IL-1β), as well as ROS and glutamate [81, 102, 103]. These neurotoxic agents elicit cellular damage and initiate the recruitment and activation of further glial cells, thus propagating the inflammatory response [81, 104]. Microglia from SOD1 mice have been shown to secrete significantly more TNFα than microglia from age-matched WT mice [99]. This is reflected in an up-regulation of TNFα mRNA and protein in the CNS of SOD1 mice [105]. Pro-inflammatory cytokines can induce COX2 expression, *via* activation of the transcription factor NF-κB [106]. COX2 activation will then increase production of prostaglandins, including $PGE_2$, which may actually contribute to excitotoxicity by stimulating the release of glutamate from astrocytes [107]. In addition, COX2 activation releases ROS, which may further propagate the inflammatory process [106].

An elevation in COX2 and NF-κB levels has been observed in post-mortem spinal cord tissue from ALS patients in reactive astrocytes [96, 108]. *In vitro,* in response to cytokine stimulation, SOD1 astrocytes produce substantially more nitric oxide (NO) than WT astrocytes [109]. NO can exert significant neuronal toxicity, *via* inhibition of mitochondrial respiration, the release of glutamate and subsequent depletion of cellular ATP [110]. Furthermore, NO reacts with superoxide radicals to generate peroxynitrite, which can nitrate tyrosine residues exerting substantial cellular damage. Accordingly, levels of 3-nitrotyrosine are elevated in motor neurons in post-mortem spinal cord tissue from ALS patients [111].

Therapeutic targeting of inflammation has been shown to be particularly effective in models of ALS. Minocycline and nordihydroguaiaretic acid (NDGA), both potent inhibitors of microglial activation and pioglitazone, an agonist of peroxisome proliferator-activated receptor γ, exert anti-inflammatory effects and significantly extend the survival of SOD1 mice [112-116]. Furthermore, selective inhibitors of COX2 delay disease onset [117] and also extend the lifespan of SOD1 mice [118]. Cannabinoids may also exert significant anti-inflammatory effects, mediated primarily by activation of $CB_2$ receptors. Indeed, cannabinoids have been shown to be neuroprotective in several experimental models of inflammation [56, 57, 102, 104, 119]. Interestingly, COX2 acts to degrade endocannabinoids [120], therefore it is possible that the neuroprotective effect of COX2 inhibitors in ALS

may be at least partly attributable to reduced degradation of endocannabinoids [121].

$CB_2$ receptors were originally regarded as peripheral cannabinoid receptors with high expression levels in the spleen and thymus [122-124]. Recently, however $CB_2$ receptors have been identified on neurons throughout the CNS, although at significantly lower levels than $CB_1$ receptors [123, 124]. $CB_2$ receptors are also expressed by microglia [81, 82, 119]. Interestingly, in response to peripheral nerve injury there is a simultaneous increase in the presence of activated microglia and the expression of $CB_2$ receptors in the rat spinal cord [125]. $CB_2$ receptor expression is also upregulated in microglia in post-mortem spinal cord tissue from ALS patients [126].

$CB_2$ receptors, like $CB_1$ receptors, are coupled to inhibitory $G_{i/o}$ proteins. Therefore, activation of $CB_2$ receptors acts to inhibit cellular activity *via* stimulation of A-type potassium channels [67]. As illustrated in Fig. (**1**), cannabinoids acting *via* a $CB_2$ receptor dependent mechanism can therefore limit the propagation of the inflammatory response by inhibiting microglial activation [127, 128] and subsequently reducing the expression and release of pro-inflammatory cytokines [81, 104]. Stimulation of microglia *in vitro* with lipopolysaccahride induces an up-regulation of TNFα and IL-1β mRNA expression in microglia [81, 104]. Treatment with cannabinoid receptor agonists, however, dose-dependently reduces the expression and release of pro-inflammatory cytokines, *via* a $CB_2$ receptor-mediated mechanism [119].

In SOD1 mice, treatment with WIN-55,212-2, a cannabinoid agonist which has a slightly higher affinity for $CB_2$ receptors than $CB_1$ receptors [129], significantly delayed the onset of motor deficits and motor neuron degeneration [87] and extended lifespan, albeit to a small extent [84] (Table **1**). Similarly, ablation of the fatty acid amide hydrolase (Faah) enzyme, which acts to hydrolyse AEA, and subsequent elevation of endocannabinoid levels [130] also delays disease progression in SOD1 mice [87]. However, it is likely that the beneficial effects of both the exogenous cannabinoid WIN-55,212-2 as well as elevated endocannabinoid levels are mediated *via* the $CB_2$ receptor. Indeed, genetic ablation of the $CB_1$ receptor in SOD1 mice has no effect on the disease symptoms and yet significantly extends their lifespan (Table **1**). This suggests that the $CB_1$ receptor may not be involved in the neuroprotective effects mediated by cannabinoids in SOD1 mice and that blockade of the $CB_1$ receptor may actually have beneficial effects. In support of this, the effects of a selective $CB_2$ receptor agonist, AM-1241, were recently examined in SOD1 mice. Functional $CB_2$ receptor expression is up-regulated in the lumbar spinal cord of SOD1 mice from a presymptomatic stage of disease and this up-regulation is maintained with further disease progression [84]. Intraperitoneal administration of the $CB_2$ receptor agonist AM-1241, which has been shown to be effective in inflammatory models [131], significantly delayed disease onset in SOD1 mice, although this effect was only seen in male mice [132], with a moderate extension in lifespan [84] (Table **1**). These results suggest that cannabinoids, acting *via* the $CB_2$ receptor, may exert therapeutic effects in ALS models most likely by inhibiting microglial activation and reducing inflammation.

## TARGETING OF OXIDATIVE STRESS BY THE ENDOCANNABINOID SYSTEM IN ALS

Several lines of evidence indicate the involvement of oxidative stress in the pathogenesis of ALS. In motor neurons in post-mortem spinal cord tissue from sporadic ALS and familial ALS patients, levels of 3-nitrotyrosine are elevated [111, 133] and in SOD1 mice, free 3-nitrotyrosine immunoreactivity and markers of lipid peroxidation are elevated even at a presymptomatic stage [97, 134]. Furthermore, evidence from SOD1 mice suggests that oxidative damage occurs to both mitochondria [135] as well as EAAT2 (GLT-1) glutamate transporter proteins [136, 137]. In contrast, there is no evidence of protein bound nitrotyrosine in either SOD1 mice or post-mortem tissue from ALS patients [134]. Similarly no increase in hydroxyl radical production can be detected in SOD1 mice [134]. Therefore, although there is evidence for oxidative damage in ALS, the mechanism by which it arises remains unknown. In familial ALS, mutations in the SOD1 enzyme structure may increase aberrant interactions with abnormal substrates such as peroxynitrite or hydrogen peroxide, or alternatively impede the binding of copper or zinc ions to the enzyme, although research in this field is conflicting (reviewed in [2]).

It is therefore possible that anti-oxidant agents that reduce oxidative stress may be an effective therapy in ALS. In 1998, Hampson and colleagues reported a potent anti-oxidant capacity of $\Delta^9$-THC and cannabidiol (CBD), *via* a cannabinoid receptor-independent mechanism, comparable to the anti-oxidant butylated hydroxytoluene. Furthermore CBD, a non-receptor binding cannabinoid, has greater neuroprotective effects following an excitotoxic insult than vitamin E, an established anti-oxidant [138]. Meanwhile, $\Delta^9$-THC and CP 55,940 show receptor-independent anti-oxidant activity *in vitro*, in response to oxidative stress mediated by serum deprivation or hydrogen peroxide exposure, respectively [55, 139]. The therapeutic benefits mediated by this anti-oxidant action of cannabinoids have yet to be established in models of ALS.

## CONCLUSIONS

At the current time the only therapy licensed for use in ALS patients is Riluzole, an anti–glutamatergic agent, which unfortunately has only limited therapeutic effects, extending patient lifespan by 2-4 months [140, 141]. In addition to excitotoxicity, several other neurotoxic pathways are implicated in ALS pathogenesis including inflammation and oxidative stress as discussed above. However, ALS is a particularly complex, multi-factorial disorder, which evidence now shows also involves deficits in axonal transport, mitochondrial damage and protein aggregation (reviewed in [2]). This multi-factorial nature of ALS pathogenesis suggests that strategies that target multiple pathways in multiple cell populations, including neurons and glia, may have greater therapeutic benefit than strategies that target individual mechanisms within specific cells types. It is becoming an increasing widely held view that "cocktail" or "combination" therapies will be necessary if a disease modifying therapy for ALS is to be developed. This proposition is supported by results that show that the combination of riluzole, minocycline, an inhibitor of microglial activation, and nimodip-

ine, a blocker of voltage-gated calcium channels, delays disease onset and extends survival in SOD1 mice to a greater extent than achieved individually with these agents [142].

In this regard, agents that are capable of modulating several pathogenic mechanisms may be particularly effective in complex neurodegenerative disorders such as ALS. Cannabinoids have been shown to exert anti-excitotoxic, anti-inflammatory and anti-oxidative effects in several experimental neurodegenerative models (for example, [51-59, 102, 104, 119, 138, 139]). In view of the involvement of these mechanisms in ALS, it is possible that agents that target the endocannabinoid system may be particularly effective in this disorder. However, although agents acting at the $CB_1$ receptor delay disease progression in SOD1 mice, it is only activation of the $CB_2$ receptor, i.e primarily targeting inflammation, which significantly extends their lifespan (Table **1**) [84, 132]. This may be related to the fact that although up-regulation of $CB_1$ receptors occurs in symptomatic SOD1 mice, this is not maintained at end-stage, in contrast to the maintained elevation in $CB_2$ receptor levels throughout disease duration [84]. Further investigation is however required to evaluate the full neuroprotective potential of agents that target the $CB_1$ receptor in order to establish whether the use of cannabinoids as a multi-targeted therapy is more effective in ALS than selective targeting of the $CB_2$ receptor. Furthermore, it will be important to establish whether the increase in endocannabinoid levels that occurs during disease progression in ALS represents an adaptive endogenous neuroprotective mechanism. To assist in this, pharmacological agents that inhibit endocannabinoid uptake (VDM11; [143]), and AEA hydrolysis (Faah inhibitor, URB597; [144]), are available. In view of the increasing body of evidence that demonstrates the neuroprotective potential of the endocannabinoid system in ALS, a full investigation of the therapeutic potential of these agents in ALS is now justified.

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## ABBREVIATIONS

2-AG = 2-Arachidonoylglycerol

AEA = Anandamide

ALS = Amyotrophic Lateral Sclerosis

AMPA = α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrate

ATP = Adenosine triphosphate

CBD = Cannabidiol

CNS = Central nervous system

COX2 = Cycloxygenase 2

EAAT2/GLT-1 = Excitatory amino acid transporter 2/glutamate transporter -1

FAAH = Fatty acid amide hydrolase

ICAM-1 = Intracellular adhesion molecule-1

IL-1β = Interleukin-1β

KA = Kainate

mGluR = Metabotropic glutamate receptors

NDGA = Nordihydroguaiaretic acid

NMDA = N-methyl D-aspartate

NO = Nitric oxide

ROS = Reactive oxygen species

SOD1 = Superoxide dismutase

TNFα = Tumour necrosis factor α

WT = Wild-type

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# REFERENCE #3

Pharmaceutical update

# Marijuana in the management of amyotrophic lateral sclerosis

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## Note

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## Abstract

*Marijuana has been proposed as treatment for a widening spectrum of medical conditions. Marijuana is a substance with many properties that may be applicable to the management of amyotrophic lateral sclerosis (ALS). These include analgesia, muscle relaxation, bronchodilation, saliva reduction, appetite stimulation, and sleep induction. In addition, marijuana has now been shown to have strong antioxidative and neuroprotective effects, which may prolong neuronal cell survival. In areas where it is legal to do so, marijuana should be considered in the pharmacological management of ALS. Further investigation into the usefulness of marijuana in this setting is warranted.*

*Key words: ALS, cannabidiol, cannabinoids, cannabinol, marijuana, symptom management*

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## Introduction

*This paper is dedicated to the memory of Linda Santos.*

Over the past few decades, there has been widening interest in the viable medicinal uses of marijuana.[1] The National Institutes of Health (NIH), the Institute of Medicine (IOM), and the Food and Drug Administration (FDA) have all issued statements calling for further investigation.[2-4] There is a large body of literature on the effects of cannabinoids on chemotherapy-induced nausea and vomiting, lowering intraocular pressure in patients with glaucoma, and treating anorexia in patients with cancer and AIDS-associated weight loss.[5-8] Beyond these clinical applications, there is limited literature describing other appropriate uses for medicinal marijuana. The intent of this article is to provide an overview of the potential pharmacological role marijuana may have in the management of amyotrophic lateral sclerosis (ALS).

To date, clinical studies on the medicinal value of marijuana have often reached differing conclusions. Some of this inconsistency in the scientific literature likely results from the fact that marijuana is a complex plant, containing over 400 chemicals.[9] Approximately 60 are cannabinoids, chemically classified as 21 carbon terpenes.[9,10] Among the most psychoactive of these is delta-9-tetrahydrocannabinol (THC).[9,10] Because of

this biochemical complexity, characterizing the clinical pharmacology of marijuana is difficult. Further complicating the evaluation of marijuana is the variable potency of the plant material used in research studies. The clinical pharmacology of marijuana containing high concentrations of THC may well differ from plant material containing small amounts of THC and higher amounts of the other cannabinoids. Moreover, the bioavailability and pharmacokinetics of inhaled marijuana are substantially different than those taken by ingestion. THC is not soluble in water, but is lipid soluble.[11] Varying proportions of other cannabinoids, mainly cannabidiol (CBD) and cannabinol (CBN), are also present in marijuana, sometimes in quantities that might modify the pharmacology of THC or have distinct effects of their own. CBD is not psychoactive, but has significant anticonvulsant, sedative, and other pharmacologic activity likely to interact with THC.[12,13] The concentration of THC and other cannabinoids in marijuana varies greatly, depending on growing conditions, plant genetics, and processing after harvest.[13] In the usual mixture of leaves and stems distributed as marijuana, concentration of THC ranges from 0.3 percent to 4 percent by weight.[13,14] However, specially grown and selected marijuana can contain 15 percent or more THC. Thus, one gram of marijuana might contain as little as 3 mg or more than 150 mg of THC.[13] THC is a potent psychoactive drug, and large doses may produce mental and perceptual effects similar to hallucinogenic drugs.[15,16] Despite this, THC and other cannabinoids have remarkably low toxicity and lethal doses in humans have not been described.[17,18]

Prior to the last decade, there was little known about the specific pharmacological and molecular effects of marijuana. However, important advances have recently taken place that have greatly increased the understanding of the receptors and ligands composing the cannabinoid system. Research has shown that two major cannabinoid receptor subtypes exist, and subtype 1 (CB1) is expressed primarily in the brain, whereas subtype 2 (CB2) is expressed primarily in the periphery.[19,20] A variety of ligands for these receptors, based on the cannabinoid structure, have been synthesized and studied. These novel ligands are of interest as both experimental tools and lead compounds for therapeutic agents. Experiments performed with several types of neural cells that endogenously express the CB1 receptor suggest the activation of protein kinases may be responsible for some of the cellular responses elicited by the CB1 cannabinoid receptor.[21] The recent discovery of the endocannabinoids, *i.e.* endogenous metabolites capable of activating the cannabinoid receptors, and the understanding of the molecular mechanisms leading to their biosynthesis and inactivation, has created a new area in research on the pharmaceutical applications of cannabinoids.[22]

The characterization of endocannabinoids, such as anandamide, and the detection of widespread cannabinoid receptors in the brain and peripheral tissues, suggests that the cannabinoid system represents a previously unrecognized, ubiquitous network in the nervous system. Cannabinoid receptors are protein-coupled, transmembrane nucleotides, similar to the receptors of other neurotransmitters such as dopamine, serotonin, and norepinephrine.[20,22] Dense receptor concentrations are found in the cerebellum, basal ganglia, and hippocampus, likely accounting for the effect of marijuana on motor tone and coordination as well as mood state.[20-22] Low concentrations are found in the brainstem, accounting for the low potential for lethal overdose.[21,22] A growing number of strategies for separating sought-after therapeutic effects of cannabinoid receptor agonists from the unwanted consequences of CB1 receptor activation are now emerging. Recently, ligands have been developed that are potent and selective agonists for CB1 and CB2 receptors as well as potent CB2-selective antagonists and inhibitors of endocannabinoid uptake or metabolism.[21,22] This knowledge may lead to the design of synthetic cannabinoid agonists and antagonists with high therapeutic potential.

## Current pharmacological management of ALS

Amyotrophic lateral sclerosis, with an incident rate of five to seven per 100,000 population, is the most common form of adult motor neuron disease.[23-26] ALS is a rapidly progressive neuromuscular disease that destroys both upper and lower motor neurons, resulting in weakness, spasticity, and ultimately death from respiratory failure. The vast majority of ALS cases are acquired and occur sporadically. Emerging evidence suggests that increased oxidative stress from free radical toxicity or excessive glutamate activity is what leads to motor neuron cell death in the brain and spinal cord.[24,27]

There is not yet a known cure for ALS, although significant research advances are being made. Riluzole is approved by the FDA for treatment of ALS.[24] This drug inhibits the presynaptic release of glutamate and reduces neuronal damage in experimental models of ALS. In 1995, two clinical trials showed that riluzole slowed disease progression.[27] Both of these studies showed prolonged survival for patients taking riluzole as opposed to placebo, although the benefit was modest. However, there are serious, but rare, complications of riluzole treatment, including renal tubular impairment, hepatitis, and pancreatitis.[27]

Because oxidative stress is one of the proposed pathogenic factors in ALS, antioxidants are recommended,

| Table 1. Properties of marijuana applicable to ALS symptom management | |
|---|---|
| **ALS symptom** | **Marijuana effect** |
| Pain | Nonopioid analgesia and anti-inflammatory |
| Spasticity | Muscle relaxant |
| Wasting | Appetite stimulant |
| Dyspnea | Bronchodilation |
| Drooling | Dry mouth |
| Depression | Euphoria |
| Dysautonomia | Vasodilation |
| Neuronal oxidation | Neuroprotective antioxidant |

including vitamin E, vitamin C, coenzyme Q, B-carotene, and N-acetylcysteine.[28,29] Creatine, an amino acid naturally found in skeletal muscle and other tissues, may also have some benefit in ALS. Creatine given to "ALS mice," a transgenic mouse model of ALS, improved motor performance, prolonged survival, and slowed loss of motor neurons.[30] At present, trials of neurotrophic factors, anti-oxidants, glutamate antagonists, and creatine are ongoing. It is currently felt that a "cocktail" approach may be the ideal treatment strategy, including glutamate antagonists, antioxidants, and neurotrophic factors.

## Application of marijuana for symptom management of ALS

Amyotrophic lateral sclerosis presents a multitude of difficult clinical problems. This section will overview these problems and discuss the potential role marijuana may play in their management. There are both direct and theoretical applications for using marijuana to manage ALS symptoms. Marijuana has easily observable clinical effects with rapid onset (*e.g.*, analgesia, muscle relaxation, dry mouth, etc.). It also has neuroprotective properties that may help prolong neuronal cell survival over extended use. This next section will delineate specific clinical problems encountered in ALS and describe the potential use of marijuana to address these.

### *Pain and immobility*

The majority of ALS patients experience significant pain.[24] The pain is due largely to immobility, which can cause adhesive capsulitis, mechanical back pain, pressure areas on the skin, and, more rarely, neuropathic pain.[24,31] Synthetic cannabinoids have been shown to produce an anti-inflammatory effect by inhibiting the production and action of tumor necrosis factor (TNF) and other acute phase cytokines.[32] Additionally, marijuana may reduce pain sensation, likely through a brainstem circuit that also contributes to the pain-suppressing effects of morphine.[33] Cannabinoids produce analgesia by modulating rostral ventromedial medulla neuronal activity in a manner similar to, but pharmacologically distinct from, that of morphine. This analgesic effect is also exerted by some endogenous cannabinoids (anandamide) and synthetic cannabinoids (methanandamide), and may be prevented by the use of selective antagonists.[34] Thus, cannabinoids are centrally acting analgesics with a different mechanism of action than opioids, although the analgesia produced by cannabinoids and opioids may involve similar pathways at the brainstem level.[35-37] Despite this promising basic science research, no clinical trials currently involving marijuana have been performed in patients with naturally occurring pain. There are two well-controlled clinical studies using marijuana in cancer pain that show significant evidence of analgesic efficacy, although these studies indicate there is a narrow therapeutic margin between the doses that produce useful analgesia and those producing euphoria and other CNS effects.[38,39]

Concern for drug overuse, within reason, is pointless in a terminal disease, and the medication should be given on a regular dosing schedule and titrated to the point of comfort.[40] Concomitant use of narcotics may also be beneficial, since the opioid receptor system appears to be separate and distinct from the cannabinoid system. In that regard, the anti-emetic effect of marijuana may help with the nausea sometimes associated with narcotic medications. Untoward effects are the possibly significant psychoactive effects of marijuana, which may include euphoria, but can also include confusion and paranoia (see *Mood state*). Some of these side effects, such as euphoria, may be quite acceptable in the final phases of life, when respiratory insufficiency or severe pain require increased doses of analgesia.[40] However, patients and caregivers should be made aware of these issues and monitor for unwanted effects.

### *Spasticity*

Spasticity in ALS is induced at

both the motor cortex and the spinal cord level through the loss of motor neuron inhibition.[24] Marijuana has an inhibitory effect on the gamma-amino-butyric acid (GABA) pathways in the central nervous system.[41] This produces motor neuron inhibition at spinal levels in mice.[42-45] Baclofen also works via the GABA pathways and would theoretically be potentiated by marijuana. Tizanidine, another commonly used anti-spasticity drug, works as an alpha-2 agonist, which is a different mechanism. Like baclofen and tizanidine, marijuana does not cause respiratory depression. This is a distinct advantage of these drugs over the benzodiazepines. Despite this, clinical evidence that marijuana relieves spinal cord spasticity is largely anecdotal. Large-scale trials or controlled studies to compare marijuana or THC with currently available therapies have not been performed and there is no published evidence that cannabinoids are necessarily superior to available therapies.

### *ALS wasting*

The term "ALS cachexia" refers to a phenomenon experienced by some patients in which weight loss occurs in excess of that caused by muscle atrophy and reduced caloric intake.[24] Both subcutaneous fat and peritoneal fat are lost, presumably because of acceleration of the basal metabolic rate.[25] In patients with ALS cachexia, greater than 20 percent of body weight is typically lost over a six-month period. Clinical studies and survey data in healthy populations have shown a strong relationship between marijuana use and increased eating.[42,46,47] Marijuana is reported to increase food enjoyment and the number of times individuals eat per day.[14,42] Mechanistic studies of marijuana on taste and satiety have shown that it does not affect taste or produce a collapse of normal satiety mechanisms.[46] Dronabinol has been shown to increase appetite and produce weight gain in AIDS and cancer patients, although the weight gain is not in lean body mass.[47] Dronabinol is approved for the treatment of anorexia in patients with AIDS-associated weight loss.[47]

### *Respiratory failure*

The terminal event in ALS is usually directly related to respiratory failure. Restrictive breathing problems usually develop in ALS and are due to weakness of the diaphragm, chest wall, and abdominal musculature.[24,25] Although cannabinoids will not likely improve respiratory muscle performance, the cannabinoids are strong bronchodilators, and pharmacologically active, aerosolized forms of THC have recently been developed.[48] This was done via a small particle nebulizer that generated an aerosol, which could penetrate deeply into the lungs. Inhalation exposure to aerosolized THC in mice elicited anti-nociceptive and bronchodilation effects that were dependent on concentration and exposure time. The anti-nociceptive and bronchodilation effects occurred within five minutes of exposure. Cannabinoid receptor antagonists, but not naloxone, blocked these effects, again indicating a cannabinoid receptor mechanism of action separate from that of the opioids.[48,49] These results demonstrate that the development of an aerosolized form of cannabinoids for human medicinal use is feasible.

### *Dysphagia*

Patients with ALS and bulbar symptoms also usually have difficulty controlling and swallowing the amounts of saliva that are normally present in the oral cavity. Marijuana is a potent antisalivatory compound that swiftly dries the oral cavity and upper airway.[48,5] Marijuana may be used alone or in conjunction with other anti-cholinergic medications to help dry up secretions. This potentially reduces the risk for aspiration pneumonia and may make the patient more comfortable.

### *Mood state*

Reactive clinical depression is expected in ALS. Marijuana will improve appetite and sleep, two problems that may be related to depression. Marijuana is often used recreationally for the "euphoria"-inducing properties, but, in some patients, it may exacerbate depression.[51] Further, it is not clear what effect marijuana will have on the pseudobulbar palsy or emotional lability of ALS. Usually, the mental and behavioral effects of marijuana consist of a sense of well-being (often termed a "high"), feelings of relaxation, altered perception of time and distance, intensified sensory experiences, laughter, talkativeness, and increased sociability when taken in a social setting.[51-53] Impaired memory for recent events, difficulty concentrating, dreamlike states, impaired motor coordination, impaired driving and other psychomotor skills, slowed reaction time, impaired goal-directed mental activity, and altered peripheral vision are commonly associated effects.[54] With repeated exposure, varying degrees of tolerance rapidly develop to many subjective and physiologic effects.[55,56] Thus, intensity of acute effects is determined not only by THC dose, but also by past experience, setting, expectations, and poorly understood individual differences in sensitivity. Large inhaled or oral marijuana doses or even ordinary doses taken by a sensitive, inexperienced, or predisposed person can produce transient anxiety, panic, feelings of depression and other dysphoric mood changes, depersonalization, bizarre behaviors, delusions, illusions, or hallucinations.[53,56] Depending on the mix of symptoms and behaviors, the state has been termed an acute panic reaction,

toxic delirium, acute paranoid state, or acute mania. These unpleasant effects are usually of sudden onset, during or shortly after smoking, or appear more gradually an hour or two after an oral dose, often lasting a few hours, and completely clear without any specific treatment other than reassurance and a supportive environment. Subsequent marijuana doses may be better tolerated.[56,57]

### *Dysautonomia*

Although dysautonomia is not generally a predominant feature of ALS, it can cause some unique clinical problems. Patients may complain of feeling quite hot, due to alterations in the autonomic control of peripheral circulation and perspiration. Marijuana produces a transient hypothermia and vasodilation, which may ease these symptoms. Skin temperature may drop four to six degrees centigrade.[50,58,59] However, marijuana is also a mild diuretic and may produce dehydration and hypotension. Thus, blood pressure and fluid intake need to be monitored in ALS patients that use marijuana and have dysautonomia.[58,59]

### *Neuroprotective and antioxidant effects*

Cannabinoids have significant neuroprotective and antioxidative effects. Recent studies have demonstrated the neuroprotective effects of synthetic, nonpsychotropic cannabinoids, which appear to protect neurons from chemically-induced excitotoxicity.[60-63] Direct measurement of oxidative stress reveals that cannabinoids prevent cell death by antioxidation. The antioxidative property of cannabinoids is confirmed by their ability to antagonize oxidative stress and consequent cell death induced by the powerful oxidant, retinoid anhydroretinol. Cannabinoids also modulate cell survival and growth of B-lymphocytes and fibroblasts.[63]

The neuroprotective actions of cannabidiol and other cannabinoids were examined in rat cortical neuron cultures exposed to toxic levels of the excitatory neurotransmitter glutamate, known to be increased in the spinal cords of ALS patients. Glutamate toxicity was reduced by both cannabidiol, a nonpsychoactive constituent of marijuana, and the psychotropic cannabinoid THC.[64] The neuroprotection observed with cannabidiol and THC was unaffected by cannabinoid receptor antagonist, indicating it to be cannabinoid receptor independent. Cannabidiol was more protective against glutamate neurotoxicity than either ascorbate (vitamin C) or alpha-tocopherol (vitamin E).[64,65]

Cannabinoids have shown efficacy as immune modulators in animal models of neurological conditions, such as experimental allergic encephalomyelitis (EAE) and neuritis.[66] These data suggest that cannabinoids might modify the presumed autoimmune cause of other neurological diseases, including multiple sclerosis (MS). Current data suggest that the naturally occurring, nonpsychotropic cannabinoid (cannabidiol) may have a potential role as a therapeutic agent for the neurodegenerative disorders produced by excessive cellular oxidation, such as ALS.

## Using marijuana

Smoking anything, including marijuana, is not healthful for the lungs and airway system.[46] Despite risk for bronchitis, the main advantage of smoking is rapid onset of effect and easy dose titration. When marijuana is smoked, THC in the form of an aerosol in the inhaled smoke is rapidly absorbed and delivered to the brain, as would be expected of a highly lipid-soluble drug.[67,68] A healthier option is vaporization. Because the cannabinoids are volatile, they will vaporize at a temperature much lower than actual combustion.[16] Heated air can be drawn through marijuana and the active compounds will vaporize, which can then be inhaled. This delivers the substance in a rapid manner that can be easily titrated to desired effect.[69] Theoretically, this removes most of the health hazards of smoking, although this has not been studied. Additionally, marijuana can be ingested orally or through a feeding tube, although oral ingestion is quite different than inhalation. The onset of action is much slower and titration of dosing is more difficult.[68,69] Maximum THC and other cannabinoid blood levels are only reached one to three hours after an oral dose.[49] The same is true of dronabinol capsules, which also have the disadvantage of containing only synthetic THC and none of the other cannabinoids.[47]

For ALS patients with severe dysphagia, the inhalation route offers additional advantages beyond rapid onset of action, particularly compared to the currently available capsule formulation. This raises many issues concerning the best mode of administration. Ideally, drug administration would be via a delivery route that is safe, easy to titrate, and readily dispersed in the body. Smoking or vaporizing plant material for inhalation poses difficulties in standardizing testing paradigms. The development of alternative dosage forms, including an inhaler form into which a controlled unit dose could be placed and volatilized would make clinical use much easier. Aerosolized cannabinoids have been developed, as described earlier, although they are not yet commercially available.

## Legal issues

An in-depth discussion of the legal ramifications of using medicinal marijuana is beyond the scope or intent of this paper. In some states, it is currently legal to use marijuana for medicinal purposes.[70-72] In Washington state, a

not-for-profit cooperative organization, the Green Cross, provides high-quality, medicinal marijuana to patients for a minimal donation, and delivers the marijuana to homebound patients. However, in other states, the use of marijuana for any purpose remains illegal. Health care providers need to know the local laws before recommending medicinal marijuana to avoid legally endangering their patients and themselves.[73-75] All decisions on the ultimate usefulness of a medical intervention should be based on a benefit/risk calculation, and marijuana is no exception to this principle.

## Conclusion

Marijuana is a substance with many properties that are directly applicable to the management of ALS. These include analgesia, muscle relaxation, bronchodilation, saliva reduction, appetite stimulation, sleep induction, and euphoria. In addition, marijuana has now been shown to have strong antioxidative and neuroprotective effects, which may prolong neuronal cell survival. From a pharmacological perspective, marijuana is reasonably safe with minimal possibility of overdose. In states where it is legal to do so, marijuana should be considered in the pharmacological management of ALS.

Moreover, the scientific process should be allowed to evaluate the potential therapeutic effects of marijuana for ALS and other disorders, detached from the societal debate over any potential harmful effects of nonmedical marijuana use.

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# REFERENCE #4

# Survey of cannabis use in patients with amyotrophic lateral sclerosis

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## Abstract

*Cannabis (marijuana) has been proposed as treatment for a widening spectrum of medical conditions and has many properties that may be applicable to the management of amyotrophic lateral sclerosis (ALS). This study is the first, anonymous survey of persons with ALS regarding the use of cannabis. There were 131 respondents, 13 of whom reported using cannabis in the last 12 months. Although the small number of people with ALS that reported using cannabis limits the interpretation of the survey findings, the results indicate that cannabis may be moderately effective at reducing symptoms of appetite loss, depression, pain, spasticity, and drooling. Cannabis was reported ineffective in reducing difficulties with speech and swallowing, and sexual dysfunction. The longest relief was reported for depression (approximately two to three hours).*

*Key words: pain, palliative care, cannabis, medicinal marijuana, amyotrophic lateral sclerosis*

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## Introduction

Amyotrophic lateral sclerosis (ALS), with an incident rate of five to seven per 100,000 population, is the most common form of adult motor neuron disease.[1-3] ALS is a rapidly progressive neuromuscular disease that destroys both upper and lower motor neurons, ultimately causing death, typically from respiratory failure. The vast majority of ALS is acquired and occurs sporadically. There is not yet a known cure for ALS.[4-6]

ALS patients may present with any number of clinical symptoms, including weakness, spasticity, cachexia, dysarthria and drooling, and pain secondary to immobility, among others.[7,8] Previous studies have reported both direct and theoretical applications for using cannabis to manage some of these ALS symptoms.[9-11] Cannabis has easily observable clinical effects with rapid onset (e.g., analgesia, muscle relaxation, dry mouth). Moreover, some components of marijuana (not inhaled smoke) have been shown in laboratory studies to have neuroprotective properties that may help prolong neuronal cell survival over extended time.[12-16]

Marijuana is a complex plant, containing over 400 chemicals.[17] Approximately 60 are cannabinoids, chemically classified as 21 carbon terpenes.[17,18] Among the most psychoactive of these is delta-9-tetrahydrocannabinol (THC).[17,18] Because of this biochemical complexity, characterizing the clinical pharmacology of marijuana is difficult. The clinical pharmacology of marijuana containing high concentrations of THC may well

differ from plant material containing small amounts of THC and higher amounts of the other cannabinoids. The bioavailability and pharmacokinetics of inhaled marijuana are also substantially different from those taken by ingestion. The cannabinoids are all lipid soluble compounds and are not soluble in water.[19] Besides THC, which is the active ingredient in dronabinol, varying proportions of other cannabinoids, mainly cannabidiol (CBD) and cannabinol (CBN), are also present in marijuana and may modify the pharmacology of THC as well as have distinct effects of their own. CBD is not psychoactive but has significant anticonvulsant and sedative pharmacologic properties and may interact with THC.[20,21]

The concentration of THC and other cannabinoids in marijuana varies greatly depending on growing conditions, plant genetics, and processing after harvest.[21] In the usual mixture of leaves and stems distributed as marijuana, concentration of THC ranges from 0.3 percent to 4 percent by weight.[21,22] However, specially grown and selected marijuana can contain 15 percent or more THC. Thus, one gram of marijuana might contain as little as three milligrams of THC or more than 150 mg.[21] THC is a potent psychoactive drug, and large doses may produce mental and perceptual effects similar to hallucinogenic drugs.[23,24] Despite this, THC and other cannabinoids have low toxicity, and lethal doses in humans have not been described.[25,26]

Despite risk for bronchitis, the main advantage of smoking is rapid onset of effect and easy dose titration. When marijuana is smoked, cannabinoids in the form of an aerosol in the inhaled smoke are rapidly absorbed and delivered to the brain, as would be expected of a highly lipid-soluble drug.[27,28] However, smoking anything, including marijuana, carries health risks for the lungs and airway system. A healthier option is vaporization. Because the cannabinoids are volatile, they will vaporize at a temperature much lower than actual combustion.[24] Heated air can be drawn through marijuana and the active compounds will vaporize, which can then be inhaled. This delivers the substance in a rapid manner that can be easily titrated to desired effect.[29] Vaporization therefore removes most of the health hazards of smoking.[27]

The medicinal use of cannabis is better documented in multiple sclerosis (MS) than in other clinical conditions, although evidence tends to be anecdotal, and no controlled clinical trials of medicinal marijuana use in MS have been published.[30-39] With respect to pain, the concomitant use of cannabis with narcotics may be beneficial, because the cannabinoid receptor system appears to be discrete from that of opioids.[40-45] In that regard, the antiemetic effect of cannabis may also help with the nausea sometimes associated with narcotic medications. Untoward effects of cannabis include potentially significant psychoactive properties, which may produce a sense of well-being or euphoria but can also induce anxiety, confusion, paranoia, and lethargy.[46]

To date there have not yet been any empirical studies to investigate the use of cannabis for medicinal purposes in ALS. The purpose of this survey was to gather preliminary data on the extent of use of cannabis among persons with ALS (PALS) and to learn which of the symptoms experienced by PALS are reported to be alleviated by the use of cannabis.

## Methodology

Participants in this survey were recruited from the ALS Digest (the Digest), an electronic discussion list published weekly to serve the worldwide ALS community, including patients, families, caregivers, and providers. The Digest serves as a forum for discussion of issues related to ALS and is not intended to provide medical advice on individual health matters. The Digest can be viewed at *www.alslinks.com*. Currently there are over 5,600 subscribers in 80 countries worldwide. However, the number of subscribers with ALS is not known. The editor is not a physician and the Digest is not peer reviewed. An e-mail invitation to participate was posted to the Digest four times over two months.

The survey was available online from January 6 through March 2, 2003, approximately eight consecutive weeks. Any subscriber with ALS was invited to participate on a voluntary and anonymous basis. The sponsoring institution human subjects review board approved the study protocol. A Web-based survey tool developed by the University of Washington was used to collect responses. The tool uses SSL encryption for transferred data, and all identifying information was stored in a code translation table separate from the actual data to protect the privacy of respondents. The University of Washington human subjects review board has approved this tool for research purposes.

PALS who wanted to participate were given a Web site address that introduced the survey and provided a link to the survey site. The invitation to participate did not mention cannabis or marijuana, in order to discourage participation by individuals who do not have ALS but might otherwise be interested in promoting legalization of marijuana. The survey was titled "A survey of ALS Patients Who Use Alternative Therapies to Treat Symptoms."

It was presumed by the investigators that the diagnostic information provided by the survey participants was accurate (i.e., no medical records were reviewed to confirm their diagnosis). In addition to a series of questions related to the ALS symptoms, the use of cannabis, and its effectiveness in

alleviating the symptoms of ALS, participants were also asked to provide demographic and diagnostic information. The survey was anonymous and it is therefore impossible to conclusively determine whether all respondents were individuals with ALS. However, the first six questions of the survey asked about how and when the respondent was diagnosed with ALS and specifically asked those who were not diagnosed with ALS by a physician to not fill out the survey. The authors carefully studied the demographic and diagnostic information provided by each respondent for completeness, consistency, and plausibility. Records with the diagnostic information missing were excluded from the analysis. Many participants offered extensive information about other alternative therapies they use, and the general comments appeared to reflect experiences of individuals living with ALS.

## Results

A total of 137 responses were received. Four responses were excluded because of duplicate submission (i.e., the same person inadvertently submitting more than one survey by hitting the submit key more than once) and two because of failure to complete most of the questions of the survey. Electronic logs of all submissions were inspected for repeated entries from the same Internet protocol (IP) address. None were found. A total of 131 responses were retained for analysis.

The demographics of the sample are shown in Table 1. Seventy-five percent of the respondents were male and 90 percent were Caucasian. The average age of participants was 54 years [standard deviation (SD) = 11], with no significant difference between the genders [Mean (M) = 54 for males, SD = 10.7; M = 53 for females, SD = 12.5]. Eighty-four percent of the respondents were married or living with a significant other, 17 percent were employed (full time or part-time), 64 percent were unemployed or retired due to disability, and 18 percent were retired due to age. Respondents reported high levels of education, with only 13 percent with high school education or less and 62 percent with college education or higher. The time since ALS diagnosis ranged from one month to 24 years. The median time since diagnosis (i.e., duration) was three years, the mean duration was approximately four years (M = 4.4, SD = 4.0). About a half of the sample reported they used a wheelchair usually or always, and about 20 percent reported no restrictions in mobility. Eighty-one percent of the respondents filled out the survey independently while 19 percent reported that they required assistance from others. One-half of the participants were taking Riluzole. The majority of participants (69 percent) reported that they live in the United States, 8 percent in Canada, and 5 percent in Australia. Six percent of the participants live in Europe, while the rest (12 percent) of the respondents reported that they were from Africa, India, Israel, Brazil, Ecuador, Guatemala, or Argentina. Fifty-three participants (41 percent) reported drinking alcohol, 14 (11 percent) reported that they used tobacco, and four (3 percent) reported consuming both alcohol and tobacco.

## Use of cannabis

Seventy-seven respondents (60 percent) reported that they never used cannabis, and 41 (31 percent) used cannabis in teenage or college years only. Thirteen respondents (10 percent) reported using cannabis in the last 12 months, and their demographics are outlined in Table 2.

Those who reported using cannabis in the last 12 months were all male and all lived in the US. Ten of those who reported using cannabis in the last 12 months also responded affirmatively to the question that asked about the use of cannabis during the teenage, college, and adult years. All of those who reported using cannabis in the last 12 months also reported that they used cannabis at some point in their lives before they were diagnosed with ALS. Six of the cannabis users reported that they lived in a state where medical cannabis is legal, and four lived in a state where medical cannabis is illegal. The remaining three respondents were not sure whether medical cannabis was legal in their state. There were no statistically significant differences between the cannabis users and non-users (see Table 2) on any demographic variable (age, marital status, employment status, education level, time since diagnosis, mobility status).

None of those who reported using cannabis in the past 12 months reported tobacco use, but all reported drinking alcohol.

Eight cannabis users reported smoking cannabis in the last three months. Two respondents reported smoking cannabis infrequently (less often than once a month), one reported smoking one to two times a week, and three reported daily use.

No respondents reported only breathing vaporized cannabis, although one participant reported using vaporized cannabis in addition to smoking and using medicinal cannabis. Two participants reported eating cannabis, one in addition to smoking it. Three respondents used medicinal cannabinoids (i.e., Dronabinol). Of the three respondents who used medicinal cannabinoids, one reported using only medicinal cannabinoids, one also smoked cannabis, and one both smoked as well as breathed vaporized cannabis.

## Symptoms

The intensity of ALS-related symptoms was quantified by asking respondents to rate how much they experience each of the symptoms on a

| Table 1. Sample demographics | | | |
|---|---|---|---|
| | | **Minimum** | **Maximum** |
| **Age** | | 25 | 82 |
| **Duration of ALS in years** | | .05 | 23.43 |
| | | **Number** | **Percent** |
| **Gender** | Male | 98 | 75 |
| | Female | 33 | 25 |
| | Total | 131 | 100 |
| **Race** | African American | 1 | 1 |
| | Asian | 4 | 3 |
| | White | 118 | 90 |
| | Other | 8 | 6 |
| | Total | 131 | 100 |
| **Employment** | Full time | 16 | 13 |
| | Part time | 5 | 4 |
| | Retired due to disability | 56 | 43 |
| | Retired due to age | 23 | 18 |
| | Unemployed due to disability | 27 | 21 |
| | Homemaker | 1 | 1 |
| | Total | 128 | 100 |
| **Education** | Less than high school | 3 | 2 |
| | High school graduate | 14 | 11 |
| | Vocational school/some college | 33 | 25 |
| | College graduate | 38 | 29 |
| | Graduate/professional degree | 42 | 33 |
| | Total | 130 | 100 |
| **Marital status** | Married/living with significant other | 110 | 84 |
| | Single/divorced/separated/widowed | 21 | 16 |
| | Total | 131 | 100 |
| **Type of ALS** | Inherited | 5 | 4 |
| | Acquired | 45 | 35 |
| | Unsure | 79 | 61 |
| | Total | 129 | 100 |
| **Mobility** | No restrictions | 27 | 21 |
| | Some difficulty walking on uneven surfaces | 16 | 12 |
| | Use canes, crutches, or walkers | 18 | 14 |
| | Usually use wheelchair | 30 | 23 |
| | Always use wheelchair | 38 | 30 |
| | Total | 129 | 100 |
| **Required assistance with filling out survey** | Yes | 25 | 19 |
| | No | 105 | 81 |
| | Total | 130 | 100 |
| **Riluzole** | Yes | 65 | 50 |
| | No | 66 | 50 |
| | Total | 131 | 100 |

| Table 2. Demographics of cannabis users | | | |
|---|---|---|---|
| | | **Minimum** | **Maximum** |
| **Age** | | 35 | 59 |
| **Duration of ALS in years** | | .8 | 10.4 |
| | | **Number** | **Percent** |
| **Gender** | Male | 13 | 100 |
| | Female | 0 | 0 |
| | Total | 13 | 100 |
| **Race** | African American | 0 | 0 |
| | Asian | 0 | 0 |
| | White | 13 | 100 |
| | Other | 0 | 0 |
| | Total | 13 | 100 |
| **Employment** | Full time | 2 | 15 |
| | Part time | 1 | 8 |
| | Retired due to disability | 6 | 46 |
| | Retired due to age | 0 | 0 |
| | Unemployed due to disability | 4 | 31 |
| | Homemaker | 0 | 0 |
| | Total | 13 | 100 |
| **Education** | Less than high school | 0 | 0 |
| | High school graduate | 1 | 8 |
| | Vocational school/some college | 4 | 33 |
| | College graduate | 6 | 51 |
| | Graduate/professional degree | 1 | 8 |
| | Total | 12 | 100 |
| **Marital status** | Married/living with significant other | 12 | 92 |
| | Single/divorced/separated/widowed | 1 | 8 |
| | Total | 13 | 100 |
| **Type of ALS** | Inherited | 0 | 0 |
| | Acquired | 6 | 42 |
| | Unsure | 7 | 54 |
| | Total | 13 | 100 |
| **Mobility** | No restrictions | 1 | 8 |
| | Some difficulty walking on uneven surfaces | 4 | 31 |
| | Use canes, crutches, or walkers | 2 | 15 |
| | Usually use wheelchair | 2 | 15 |
| | Always use wheelchair | 4 | 31 |
| | Total | 13 | 100 |
| **Required assistance with filling out survey** | Yes | 2 | 15 |
| | No | 11 | 85 |
| | Total | 13 | 100 |
| **Riluzole** | Yes | 4 | 31 |
| | No | 9 | 69 |
| | Total | 13 | 100 |

| Table 3. Symptoms reported by cannabis users | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Symptom | Cannabis nonusers | | | | | Cannabis users | | | | |
| | Minimum | Maximum | Mean intensity | SD | N | Minimum | Maximum | Mean intensity | SD | N |
| Weakness | 0 | 4 | 2.87 | 1.05 | 118 | 1 | 4 | 2.85 | 0.99 | 13 |
| Speech difficulties | 0 | 4 | 1.93 | 1.55 | 118 | 1 | 4 | 2.22 | 1.09 | 9 |
| Drooling | 0 | 4 | 0.97 | 1.15 | 118 | 1 | 4 | 2.00 | 1.15 | 7 |
| Swallowing difficulties | 0 | 4 | 1.36 | 1.33 | 118 | 1 | 4 | 2.00 | 1.20 | 8 |
| Shortness of breath | 0 | 4 | 1.11 | 1.14 | 118 | 1 | 4 | 1.80 | 1.03 | 10 |
| Pain | 0 | 4 | 1.14 | 1.15 | 118 | 1 | 4 | 1.67 | 1.21 | 6 |
| Spasticity | 0 | 3 | 1.77 | 1.25 | 118 | 1 | 3 | 1.63 | 0.92 | 11 |
| Appetite loss | 0 | 3 | 0.73 | 1.06 | 118 | 1 | 3 | 1.56 | 0.88 | 9 |
| Depression | 0 | 3 | 1.20 | 1.07 | 118 | 1 | 3 | 1.50 | 0.85 | 10 |
| Sexual dysfunction | 0 | 2 | .97 | 1.38 | 118 | 1 | 2 | 1.25 | 0.50 | 4 |

five-point scale ranging from "not at all" (0) to "very much" (4). The most frequent symptom was weakness (reported by all cannabis users). The mean intensity was highest for weakness, followed by speech difficulties, drooling, and swallowing difficulties. The intensity of symptoms reported by respondents who did not use cannabis was not statistically significantly different from the symptom intensity reported by the cannabis users [$F(10, 120) = 1.07$, $P = .39$]. A summary of symptoms and their intensity is listed in Table 3.

The amount of relief attributed to cannabis use was assessed by asking the respondents to rate the degree to which cannabis alleviates each symptom on a five-point scale ranging from "not at all" (0) to "completely relieves the symptom" (4). Respondents reported that the use of cannabis helped moderately for depression, appetite loss, spasticity, drooling, and pain. All cannabis users who reported symptoms of appetite loss and depression also reported that cannabis reduced these symptoms. None of the cannabis users reported any reduction in difficulties with swallowing and speech or sexual dysfunction.

The duration of symptom relief was measured on a scale from 0 (no relief) to 6 (more than nine hours). Respondents reported the most lasting relief (on average two to three hours) for depression. The loss of appetite, drooling, shortness of breath, spasticity, and pain were reported to be relieved on average for approximately one hour or less. Table 4 provides a summary of symptoms reported by the cannabis users. Level of relief was reported on a five-point scale ranging from "not at all" (0) to "completely relieves the symptom" (4). The duration of symptom relief was measured on a scale from "no relief" (0), "less than one hour" (1), "two to three hours" (2), "four to five hours" (3), "6 to 7 hours" (4), "eight to nine hours" (5), "more than nine hours" (6).

## Discussion

There is an increasing amount of research concerning the medicinal effects of cannabinoids. For example, cannabinoids have been reported to reduce chemotherapy-induced nausea and vomiting, lower intraocular pressure in patients with glaucoma, reduce anorexia in patients with cancer and AIDS-associated weight loss, and reduce pain and spasticity in MS.[30-39] Cannabinoids, the active ingredients in marijuana, may also have properties that may be applicable to the management of ALS.[9,10] However, to date no empirical studies of use and effectiveness of cannabis for symptom management by PALS have been published.

Approximately 10 percent of the survey respondents reported using cannabis. This is a lower rate than the

| Table 4. Level and duration of relief following use of cannabis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Symptom** | **Minimum** | **Maximum** | **Average relief** | **SD** | **Reported no relief** | **Percent** | **Reported some relief** | **Percent** | **Total N** |
| **Weakness** | | | | | | | | | |
| degree of relief | 0 | 2 | 0.75 | 0.71 | 3 | 37.50 | 5 | 62.50 | 8 |
| length of relief | 0 | 3 | 1.60 | 1.14 | | | | | 5 |
| **Speech difficulties** | | | | | | | | | |
| degree of relief | 0 | 0 | 0.00 | 0.00 | 6 | 100.00 | 0 | 0.00 | 6 |
| length of relief | | | | | | | | | |
| **Drooling** | | | | | | | | | |
| degree of relief | 0 | 3 | 1.75 | 1.25 | 1 | 25.00 | 3 | 75.00 | 4 |
| length of relief | 2 | 2 | 2.00 | 0.00 | | | | | 3 |
| **Swallowing difficulties** | | | | | | | | | |
| degree of relief | 0 | 0 | 0.00 | 0.00 | 6 | 100.0 | 0 | 0.00 | 6 |
| length of relief | | | | | | | | | |
| **Shortness of breath** | | | | | | | | | |
| degree of relief | 0 | 3 | 0.60 | 1.34 | 4 | 80.00 | 1 | 20.00 | 5 |
| length of relief | 2 | 2 | 2.00 | 0.00 | | | | | 1 |
| **Pain** | | | | | | | | | |
| degree of relief | 0 | 3 | 1.67 | 1.21 | 1 | 16.67 | 5 | 83.3 | 6 |
| length of relief | 1 | 3 | 2.00 | 0.71 | | | | | 5 |
| **Spasticity** | | | | | | | | | |
| degree of relief | 0 | 4 | 1.86 | 1.46 | 2 | 28.57 | 5 | 71.43 | 7 |
| length of relief | 1 | 3 | 2.00 | 0.71 | | | | | 5 |
| **Appetite loss** | | | | | | | | | |
| degree of relief | 1 | 4 | 2.13 | 1.13 | 0 | 0.00 | 8 | 100.00 | 8 |
| length of relief | 2 | 3 | 2.17 | 0.40 | | | | | 6 |
| **Depression** | | | | | | | | | |
| degree of relief | 1 | 4 | 2.13 | 1.13 | 0 | 0.00 | 8 | 100.00 | 8 |
| length of relief | 1 | 4 | 2.67 | 1.03 | | | | | 6 |
| **Sexual dysfunction** | | | | | | | | | |
| degree of relief | 0 | 0 | 0.00 | 0.00 | 3 | 100.00 | 0 | 0.00 | 3 |
| length of relief | | | | | | | | | |

frequency of use reported by other patient populations, including MS, AIDS, and cancer patients.[10,30,31] However, the pattern of symptom relief reported by the small number of PALS who reported using cannabis[13] was consistent with the reported effects of cannabis for symptom management by people with other conditions, including MS.[30,35,36] Cannabis users reported that cannabis smoking was most effective at reducing depression, appetite loss, pain, spasticity, drooling, and weakness. The factor that most predicted current use of cannabis by PALS was reported previous use (presumably recreational).

The survey had a number of limitations. First, the survey results reported here are based on a relatively small number of respondents (131) and on reports of 13 cannabis users, and may not be representative of the patterns of cannabis use in the ALS population by people with ALS in general. Second, 75 percent of the respondents were male, 25 percent were female. Men appear to be about 1.5 times more likely to be affected with ALS than women,[7,8] so the percentage of female participants is slightly lower than expected in the general ALS population (about 33 percent). Published studies of Internet use consistently report that females are less likely to use the Internet for reasons that may be independent of income and estimate that only about one-third of Internet users are women.[47,48] This may account for the lower than expected participation by women with ALS.

A third limitation of the study is that a disproportionate number of the survey respondents were white (90 percent) and all cannabis users were white. There is some evidence that whites may be at higher risk for ALS, though most researchers agree that ALS equally affects people of all races.[49,50] Racial discrepancies in rates of ALS may be due to poorer access to healthcare for minority populations in the US, particularly access to tertiary referral centers, where the ALS diagnosis is often made. Published studies report that over 80 percent of Internet users are white;[48] this is the most likely explanation for the disproportionate participation by Caucasians in this survey.

Fourth, Internet users tend to be highly educated. Almost 60 percent report having at least one degree.[48] Those with higher education are more likely to own computer equipment and to use it to connect to the Internet.[51] The results of the survey we report here provide further evidence for this trend, with only 13 respondents (10 percent) reporting having high school education or less.

Finally, none of the participants from the countries where cannabis use is prevalent (India) or legal for medical uses (Australia, Canada) reported using cannabis. The most likely explanation for this finding is the small number of participants from these countries; only one respondent was from India, six from Australia, and eleven from Canada.

In general, professionals with university degrees living in households with disposable incomes sufficient to purchase technology tools are likely to be over-represented in Internet surveys. Women, minorities, the elderly, those who live on social assistance disability payments, or who earn minimum wages, are much less likely to participate.[48,51]

Privacy is a major issue associated with Web-based methodology. When the Internet is used for research, especially for research on sensitive issues (such as using substances that are illegal under federal law and most state laws), protecting the privacy of the participants is paramount. By making the survey anonymous, the authors protected the privacy of the respondents but gave up the ability to verify respondents' diagnoses or prevent repeated or malicious submittals. Although the records showed that no two responses were submitted from the same IP address, the IP address identifies the computer, not the user. Therefore, it cannot be conclusively determined that one respondent did not submit more than one response using different computers.

The low response rate might be explained by many factors. First, we do not know how many participants in the electronic discussion list that was used to recruit participants have ALS. It is possible, even likely, that a large majority of the participants are family members, service providers, and advocates. Second, the respondents who do not use alternative therapies may have been less likely to respond. It is unclear what percentage of people with ALS use alternative therapies. A recent survey from Germany suggests that about half of the ALS patients there use complementary and alternative medicine.[52] Some respondents who do not use alternative therapies such as vitamins and supplements, but do use cannabis to manage their symptoms may not have considered cannabis to be an "alternative therapy" and decided not to participate. Many respondents provided information on vitamins, supplements, and other alternative therapies in the write-in spaces of the survey even though they were not asked about these therapies directly, probably because the respondents had anticipated the survey would gather information on those topics. Third, even though the invitation as well as the introduction to the survey clearly stated that the survey was anonymous and there was no way for the researchers to associate a specific response with a specific respondent, many may have been individuals who are generally suspicious of providing information via the Internet and may have decided not to participate for this reason.

Despite the limitations of this study noted above, these preliminary findings support the need for further

research into the potential benefits of cannabis use for the clinical management of some ALS symptoms. These include pain, which was one of the symptoms identified in a recent study as not being sufficiently addressed in ALS.[53] Further research is needed to see if the current findings can be confirmed using non-Internet-based survey methodology with a defined sample. It would also be informative to inquire about cannabis use within the context of subject beliefs about the efficacy of various alternative and complimentary approaches and their engagement and satisfaction with those approaches.

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# REFERENCE #5

# Cannabidiol: An Overview of Some Pharmacological Aspects

*Raphael Mechoulam, Linda A. Parker, and Ruth Gallily*

*Over the past few years, considerable attention has focused on cannabidiol (CBD), a major nonpsychotropic constituent of cannabis. The authors present a review on the chemistry of CBD and discuss the anticonvulsive, antianxiety, antipsychotic, antinausea, and antirheumatoid arthritic properties of CBD. CBD does not bind to the known cannabinoid receptors, and its mechanism of action is yet unknown. It is possible that, in part at least, its effects are due to its recently discovered inhibition of anandamide uptake and hydrolysis and to its antioxidative effect.*

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Cannabidiol (CBD) was first isolated from the cannabis plant in the late 1930s and early 1940s, and its structure was elucidated in 1963. For an introduction to the chemistry of CBD, see Mechoulam and Hanus.[1] No pharmacological work was reported on CBD until the early 1970s, except the determination that it had no cannabis-like activity in vivo.[2,3] Over the next few years, some work was reported, particularly on its anticonvulsive effects. Later, antianxiety effects were noted, and some of its actions on the immune system were explored. More recently, its effects on nausea, as an antioxidant in biological systems and as an antirheumatoid arthritis drug, were reported. The present review summarizes these advances. Zuardi et al[4] have recently critically discussed the effects of CBD on some of these states. To avoid duplication, we emphasize in this review the antinausea and immune system effects, including rheumatoid arthritis, that are not evaluated by Zuardi et al.

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## CBD: ANTICONVULSIVE EFFECTS

In the early 1970s, several groups found that CBD was active in reducing or blocking convulsions produced in experimental animals by a variety of procedures.[5-7] The CBD effects were comparable to those of diphenylhydantion (DPH) and other drugs, which are clinically effective in major seizures.[8] CBD was also found to enhance the anticonvulsant potency of DPH and phenobarbital.[8-9] Karler and Turkanis[10] compared the effects of CBD and THC in the maximal electroshock test in mice, which measures anticonvulsant activity. The $ED_{50}$ of CBD (118 mg/kg) was close to that of $\Delta^9$-THC (101 mg/kg). In frogs (*Rana pipiens*), both cannabinoids were about 1000 times more active, but only in the summer. In the winter, the frogs were not responsive to either cannabinoid, even at massive doses.[10] However, in another assay—the pentylene tetrazol minimal-seizure threshold test in mice—differences between the activities of CBD and THC were noted. It was assumed that THC and CBD act by different mechanisms, with CBD more closely resembling the well-established antiepileptics at that time (e.g., phenobarbital and DPH) than does $\Delta^9$-THC. Indeed, when conformational energy maps were computed and compared for DPH and CBD, it was noted that the spatial relationship between the two rings in the two drugs was similar and close to the respective structures in the crystal. This was supported by $^1H$ and $^{13}C$ NMR measurements. It was also found that both compounds ful-

fill the stereochemical requirements suggested for anticonvulsant drug action.[11]

The early preclinical anticonvulsant work is well reviewed.[12,13] Consroe,[14] in a more recent review, has suggested that CBD is largely inactive in animal models of absence seizures produced by electroshock or chemoshock models. However, it is active against cortical focal seizures produced by topical application of convulsant metals or limbic seizures produced by electrical stimulation or kindling, as well as in generalized maximal (tonic-clonic) seizures produced by electroshock or GABA-inhibiting drugs.

Both CBD enantiomers are anticonvulsive.[15] It is quite possible that they act by different mechanisms. While the natural (–) CBD does not bind to the central cannabinoid receptor, CB1, the synthetic (+) CBD has recently been shown to bind to CB1.[16] The mechanism of (–) CBD anticonvulsive activity is unknown; however, it is reasonable to assume that (+) CBD, like THC, acts by activation of CB1. Recently, Wallace et al[17] compared the anticonvulsant effects of THC with those of CBD. The effects of THC could be blocked with a cannabinoid receptor antagonist, while those of CBD could not. The authors thus confirmed that the effects of CBD are not CB1 receptor mediated. These conclusions support the early observation by Karler and Turkanis.[10]

CBD has very low toxicity. $LD_{50}$ on IV administration to the rhesus monkey was 212 mg/kg.[18] The oral $LD_{50}$ could not be established, but it was pointed out that "the results obtained with prolonged oral CBD treatment should be viewed with the knowledge that the oral route requires 20-50 times larger cannabinoid dose than the i.v. route to initiate severe intoxication."[18] CBD did not elicit signs of CNS inhibition or stimulation and did not cause autonomic aberrations. Clinical measurements, eye examinations, and EKG recordings were normal. There were no significant alterations in growth rates.

The pharmacokinetics of CBD is quite complicated.[19] On IV administration, CBD is rapidly distributed, followed by prolonged elimination (terminal half-life = 9 h). CBD is barely absorbed after oral administration. The oral bioavailability ranges between 13% and 19%, which may be due to a first-pass effect. These observations may explain the results, described above, by Rosenkrantz et al.[18]

The essential lack of toxicity made possible an early anticonvulsive clinical trial.[20] After a phase I clinical trial in healthy volunteers, 15 patients suffering from secondary generalized epilepsy with temporal focus were randomly divided into two groups. Each patient received, in a double-blind procedure, 200 to 300 mg daily of CBD or placebo. The drugs were administered for as long as 4½ months. Clinical and laboratory examinations, EEG, and ECG were performed at 15- or 30-day intervals. Throughout the experiment, the patients continued to take the antiepileptic drugs prescribed before the experiment, although these drugs no longer controlled the signs of the disease. All patients and volunteers tolerated CBD very well, and no signs of toxicity or serious side effects were detected on examination. Four of the 8 CBD subjects remained almost free of convulsive crises throughout the experiment, and 3 other patients demonstrated partial improvement in their clinical condition. CBD was ineffective in 1 patient. The clinical condition of 7 placebo patients remained unchanged, whereas the condition of 1 patient clearly improved. Due to the huge amounts of drug required, this promising clinical trial was never continued.

## CBD: SEDATIVE AND ANXIOLYTIC EFFECTS

In the early 1980s, several groups independently discovered that CBD has sedative and antianxiety properties, albeit at doses higher than those of the clinically used drugs at that time. Pickens[21] compared THC and CBD with chlorpromazine administered orally to mice and found that the sedative potency ($SD_{50}$) was 1.06 mg/kg for THC, 1.26 mg/kg for chlorpromazine, and 4.72 mg/kg for CBD.[21]

Musty[22] found that CBD improved avoidance learning in a stressful situation, decreased the occurrence of stress-induced ulcers in mice, and decreased response suppression in a punished response task. In a further work, Musty et al[23] showed that CBD affects conditioned anxiety-like behavior in a taste aversion model.

A Brazilian group, on the basis of initial studies in rats (unfortunately, some published in Portuguese and not generally available), undertook an evaluation of the action of CBD on anxiety and other effects produced by THC in normal subjects.[24] They found that CBD blocks the anxiety produced by THC. This effect also extended to other CNS effects caused by THC; however, not all THC effects were blocked. The effect of THC on pulse rate was unchanged. These observed effects support the widely held view that cannabis effects differ from those of THC alone, as the crude drug contains both CBD and THC. The same group later compared the anxiolytic effects of CBD with those of ipsapirone (a $5HT_{1A}$ partial agonist) and with diazepam in a double-blind study in a simulated public speaking test.[25] All three compounds were active, although the doses of CBD needed were considerably higher than those of the

two other drugs. No further human trials with CBD in anxiety have been reported, and there are no publications directly comparing the action of CBD, CBD/THC, or THC/cannabis in humans. However, the results in humans have been confirmed in animal studies. Guimaraes et al[26] showed that CBD in doses of 2.5, 5.0, and 10.0 mg/kg significantly increased the entry ratio (open/total number of entries) in the elevated plus-maze assay, an anxiolytic-like effect. CBD at a dose of 20.0 mg/kg was not effective. These results indicate that the anxiolytic effect of CBD in the elevated plus-maze, like many other effects of cannabinoids, is biphasic (cf. Sulcova et al[27]).

In a further publication by the same group, it was shown that the dimethylheptyl homolog of CBD (HU-219) is considerably more potent than CBD or diazepam in the same assay.[28]

Onaivi et al[29] also reported that, in contrast to effects seen with THC, mice treated with CBD spent a greater amount of time in the open arm of the elevated plus-maze, an effect similar to that produced by diazepam, the reference anxiolytic agent.

## CBD: HYPNOTIC EFFECT

Monti[30] has reported that 20 mg/kg single doses of CBD decreased slow-wave sleep latency in rats, but higher doses caused an increase. However, wakefulness was decreased. This is another example of the biphasic action of CBD.

Carlini and Cunha[31] reported that relatively high doses of CBD (160 mg) caused significantly longer sleep in insomniacs than those on placebo.

## CBD: ANTIPSYCHOTIC EFFECTS

Zuardi et al[32] have shown that CBD is active in animal models predictive of antipsychotic activity. Thus, CBD (15-480 mg/kg) reduced the occurrence of stereotype behavior induced by apomorphine and increased the doses of apomorphine needed to cause such behavior. The same effects were observed with haloperidol, albeit at much lower doses. However, haloperidol caused catalepsy at high doses, while CBD did not.

On the basis of these preclinical experiments and lack of toxicity (see above), a single case clinical trial was undertaken: a young 19-year-old black woman, diagnosed as schizophrenic, was administered CBD (up to 1.5 g/day). Improvement with CBD was observed in all items of the standard Brief Psychiatric Rating Scale (BPRS) and was essentially equivalent to that seen with haloperidol. The authors concluded that CBD may possess an atypical antipsychotic profile.[33]

A German group has looked into the effects of nabilone (a synthetic cannabinoid drug with THC-like properties) and CBD on binocular depth inversion. This visual phenomenon is a normal illusion of visual perception and is reduced in schizophrenic patients.[34] While nabilone caused impairment of binocular depth inversion, CBD reduced this impairment.[35] On this basis, the same group administered CBD to schizophrenic patients. Preliminary results, presented at a meeting, indicate positive results.[36] Is CBD, or a more potent derivative, going to become a new antischizophrenic drug?

## CBD: ANTI-INFLAMMATORY EFFECTS

The pathogenesis involved in inflammatory reactions is complex and multifunctional. It is triggered and maintained by various intercellular mediators—the cytokines. One of these cytokines, tumor necrosis factor (TNF), is particularly important in triggering a cascade of other cytokines, which also participate in the inflammatory process. The rise and involvement of TNF in many pathological manifestations are well established. Recently, very encouraging results using anti-TNF therapy for rheumatoid arthritis and colitis were reported.[37] Potent suppression of the clinical manifestations of these chronic diseases was noted.

It is well established that stimulation causes a respiratory burst in phagocytes, characterized by a sharp increase in oxygen uptake. Reactive oxygen intermediates (ROI) are formed whose antimicrobial and antitumor activity is of major importance in the protection of body systems.[38]

Nitric oxide (NO) is an endogenous modulator with diverse biological functions.[39] It is produced by most mammalian cells and mediates multiple physiological and pathological processes. For example, it is a major endogenous regulator of vascular homeostasis and serves as a neurotransmitter in the brain and other parts of the body. NO has also been shown to possess antibacterial and antitumor activity[40] and affects various aspects of the inflammatory cascade.

It is well known, however, that many weapons of the immune system, which have the capacity to eliminate microbes and tumors, can also harm the host. For example, high levels of TNF, ROI, and NO can cause inflammation and damage cells and tissues and may also contribute to septic shock. Therefore, a primary therapeutic goal of using drugs acting on the immune system is to limit the effects of TNF, ROI, and NO.

A vast literature documents the immune-modulating effects of cannabinoids, mainly of $\Delta^9$-THC, in vivo and

in vitro.[41] A partial list of in vitro effects of $\Delta^9$-THC includes inhibition of the proliferative responses of T lymphocytes, inhibition of cytotoxic T cell activity, suppression of macrophage function and antigen presentation, and inhibition of NO production by macrophages. CBD has been reported to cause modulation of TNF, IL-1, and IFN-γ production by human peripheral blood mononuclear cells.[42,43] It suppresses chemokine production by a human B cell line.[44] These potentially anti-inflammatory properties of CBD, together with the lack of psychotropic effects and low toxicity, prompted Malfait et al[45] to test the potential of CBD as a therapeutic agent in collagen-induced arthritis (CIA).

CIA is a murine model for rheumatoid arthritis (RA). It is elicited by immunizing mice with type II collagen (CII) in complete Freund's adjuvant. The CII used is either bovine or murine, resulting in classical acute CIA or in chronic relapsing CIA, respectively. CBD was administered after onset of clinical symptoms, and in both models of arthritis, the treatment blocked progression of the disease.[45] CBD was effective when administered either i.p. or orally. The dose dependency showed a bell-shaped curve, with an optimal effect at 5 mg/kg per day i.p. or 25 mg/kg per day orally. Ex vivo, draining lymph node cells from CBD-treated mice showed diminished IFN-γ production, as well as decreased release of TNF by knee synovial cells. In vitro effects of CBD included a dose-dependent suppression of lymphocyte proliferation and the blockade of the Zymosan-triggered reactive oxygen burst generation by peritoneal granulocytes. CBD markedly lowered the production of TNF and NO in vitro by peritoneal macrophages (our unpublished data). It also suppressed mouse lymphocyte responses to mitogens and to allogenic stimuli and blocked the lipopolysaccharide-induced rise in serum TNF in mice. Taken together, these data show that CBD, through its combined immunosuppressive and anti-inflammatory actions, has a potent antiarthritic effect in CIA.[45]

## CBD: ANTINAUSEA EFFECTS

The development of chemotherapy treatment has prolonged the lives of many cancer patients. However, use of these powerful drugs presents a serious challenge to both clinicians and patients. Significant side effects of cancer chemotherapy include nausea and vomiting, which may last for several days. These symptoms come to be dreaded by patients, often interfering with successful completion of treatment.

Although nausea often occurs prior to vomiting, each can occur independently. Nausea is a subjective phenomenon, an "unpleasant, but not painful, sensation associated with a heightened awareness of the upper gut, cold sweating and the feeling that vomiting is imminent."[46] In contrast, vomiting is a far more visible and easily recorded indictor of chemotherapy-induced side effects. In fact, vomiting is used in some antiemetic trials as the sole criterion of efficacy.[47] However, drugs effective against vomiting may not necessarily modify nausea, and drugs effective against nausea may not necessarily modify vomiting.

### THC as an Antiemetic

Testimony of numerous patients, including the late Steven Jay Gould, indicates that marijuana reduces nausea and vomiting associated with chemotherapy, thereby maintaining the resolve to continue with therapy.[48] A survey of more than a thousand cancer specialists found that 44% had recommended THC or cannabis to at least one patient.[49]

Treatment of nausea is one of the few medical uses of a marijuana constituent that has been evaluated with clinical trials. The results of these trials, conducted primarily in the 1970s, indicated that pure $\Delta^9$-THC and the synthetic cannabinoid nabilone (an analogue of THC) were as effective as any other antinausea agent available at the time.[50,51] For a review of clinical trials with $\Delta^9$-THC (dronabinol), see Mechoulam et al.[52] There have been no animal or clinical trials that compare the effectiveness of cannabinoids with the powerful antiemetic $HT_3$ antagonists, nor have there been trials that evaluate cannabinoid use in combination with the serotonin antagonists.[53]

Recent experimental evidence that marijuana interferes with nausea and vomiting is limited. THC eliminates vomiting produced by cisplatin and the cannabinoid receptor antagonist SR 141716A.[54,55] The mechanism of action of the antinausea properties of THC is unknown; however, THC has been reported to reverse the effects of 5-$HT_3$ receptor agonists (which induce vomiting) in the nucleus tractus solitarii at the level of the area postrema, the chemoreceptor trigger zone for emetic reflexes.[56]

### Conditioned Rejection Reactions as a Rat Model of Nausea

Animal models are essential to examine the efficacy and safety of agents used to treat the distressing side effects of both nausea and vomiting. Furthermore, animal models provide the opportunity to experimentally manipulate cues associated with chemotherapeutic

agents and to evaluate antiemetic treatment for anticipatory nausea and vomiting.

The phenomenon of nausea has been assessed exclusively by self-report in humans. However, there is considerable evidence (reviewed below) that nausea[57,58] and conditioned nausea[59,60] are displayed in rats as rejection reactions. The association between the flavor and activation of the emetic system results in altered affective reactions to the food or fluid. These altered affective reactions are called conditioned rejection reactions (gaping, chin rubbing, and paw treading in the taste reactivity test devised by Grill and Norgren[61]). Conditioned rejection reactions are exclusively elicited by emetic agents.[59,60,62-65]

Recent work indicates that conditioned rejection reactions in the taste reactivity test predict the emetic properties of an agent (in species that are capable of vomiting).[57,59-60] Since rats are incapable of vomiting, we have argued that these conditioned rejection reactions reflect nausea, based on the following evidence: (1) conditioned rejection reactions are selectively elicited by emetic treatments, such as lithium chloride, cyclophosphamide, high doses of nicotine, high doses of apomorphine, and full body rotation.[59,60,66] (2) Antiemetic treatments, including 5-HT antagonists and cannabinoid agonists, attenuate these conditioned rejection reactions.[67-70] (3) The literature on conditioned flavor avoidance learning has shown that flavor avoidance produced by drugs that elicit vomiting in other species is mediated by their action on the emetic systems of the midbrain and brainstem in rats. Ablation of the area postrema selectively eliminates taste avoidance and behavioral evidence of sickness produced by emetic agents.[71-74] (4) Ablation of the area postrema eliminates toxin-induced conditioned rejection reactions.[57,75] (5) Grundy[76] and his colleagues report that the vagal response to electrical and chemical stimulation by cytotoxic drugs in rats is similar to that of ferrets,[77-79] a species that vomits in response to these stimuli. This neural afferent reaction is disrupted by 5-$HT_3$ antagonists in both ferrets and rats.[75] These findings indicate that the gastrointestinal signals that precede vomiting in ferrets also occur in rats, suggesting that both species experience nausea.

### Effect of Cannabinoids on Conditioned Rejection Reactions

We have recently reported that a low dose (0.5 mg/kg) of THC also attenuates conditioned rejection reactions,[68] although a much higher dose (2.5 mg/kg) is aversive to rats.[80] Limebeer and Parker[68] found that a dose of 0.5 mg/kg of THC eliminates the establishment of conditioned rejection reactions and the expression of previously established conditioned rejection reactions elicited by a cyclophosphamide-paired flavor. Cyclophosphamide is an agent used in chemotherapy treatment in humans. Rats administered THC during conditioning and during testing also displayed suppressed conditioned rejection reactions. Therefore, the decrement in responding at testing cannot be attributed to a change in state from conditioning to testing (i.e., our results cannot be attributed to state-dependent learning). These results demonstrate that THC interferes with cyclophosphamide-induced nausea in rats during conditioning and with conditioned nausea (anticipatory nausea) during testing.

## CANNABIDIOL INTERFERES WITH NAUSEA IN RATS

Both THC (generic name dronabinol) and nabilone are clinically approved antinausea drugs for human patients, but as mentioned above, many users claim that marijuana suppresses nausea more effectively than oral THC.[48] In fact, the psychoactive effects of THC are disturbing to some patients, causing termination of use even though it may be effective against nausea.

Parker et al[70] evaluated the potential of CBD, which as mentioned above does not produce psychoactive effects, and its synthetic dimethylheptyl homolog (CBD-DMH) to suppress nausea in the conditioned rejection model. The potential of these nonpsychoactive cannabinoids to interfere with nausea was determined by administering them prior to lithium on the conditioning trial. In this trial, rats were injected with a low dose (5 mg/kg i.p.) of CBD, CBD-DMH, or vehicle 30 minutes prior to a pairing of saccharin solution and lithium chloride (20 ml/kg of 0.15 M LiCl) or saline. The potential of CBD and CBD-DMH to interfere with the expression of a previously established conditioned rejection (a model of anticipatory nausea) was evaluated by administering them prior to exposure on the taste reactivity test trial. On each of two tests, rats were injected with 5 mg/kg i.p. of the test drug (CBD, Experiment 1; CBD-DMH, Experiment 2) on one trial and with the vehicle on the other trial (in a counterbalanced order) 30 minutes prior to an intraoral infusion of saccharin solution. The rejection reactions (gapes, chin rubs, and paw treads) displayed by the rats during the infusion were videotaped.

Figure 1 presents the mean frequency of summed rejection reactions displayed during both the vehicle test trial and the drug test trial for each experiment. The pattern of results in both experiments was identical. Group vehicle lithium displayed conditioned rejection

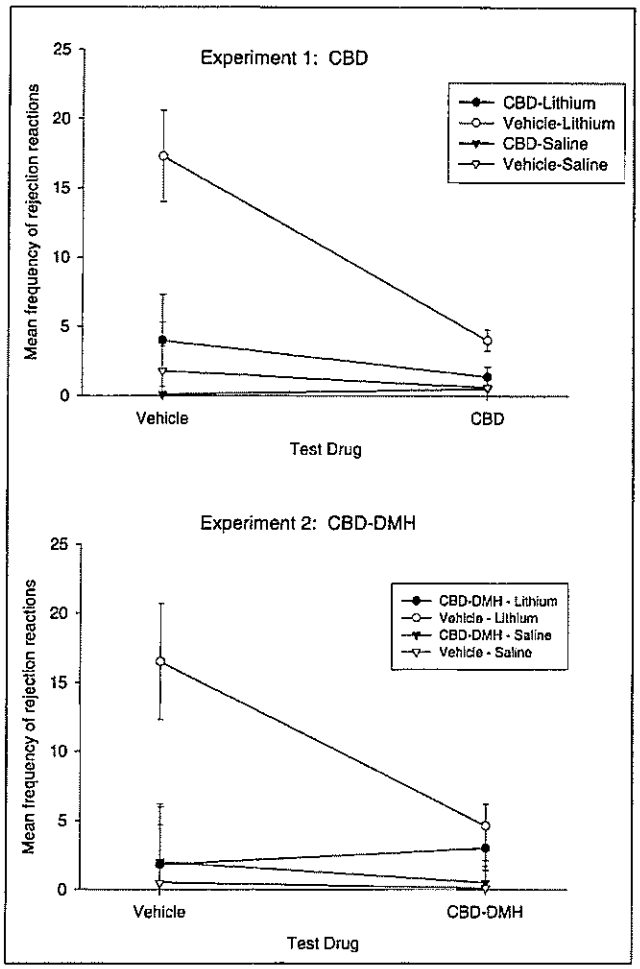


*Figure 1. Mean (±SEM) frequency of conditioned rejection reactions displayed by groups pretreated with CBD (Experiment 1) or CBD-DMH (Experiment 2) during conditioning and testing. On the conditioning trial, independent groups received the cannabinoid or vehicle prior to receiving an intraoral infusion of saccharin solution, which was immediately followed by lithium or saline. On each of two test trials, rats were injected with the cannabinoid or vehicle (counterbalanced order) prior to receiving an intraoral infusion of saccharin solution.*

reactions during the vehicle test only. When either CBD (Experiment 1) or CBD-DMH (Experiment 2) preceded lithium during conditioning, no rejection of saccharin solution occurred during the drug-free test (presumably because the drug interfered with lithium-induced nausea). Furthermore, when rats were administered either CBD or CBD-DMH prior to the test for conditioned rejection, these reactions were suppressed (presumably because the drug interfered with conditioned nausea).

These results suggest that the nonpsychoactive component of marijuana, CBD, and its synthetic homolog, CBD dimethylheptyl, interfere with nausea and conditioned nausea in rats. They provide promise for the development of an effective antinausea cannabinoid treatment for chemotherapy-induced nausea that is devoid of psychoactive side effects.

## CBD: SPECULATIONS ON ITS MECHANISM OF ACTION

We have described above various effects caused by CBD. However, we are quite ignorant as to the biochemical or physiological mechanisms that are the basis of these activities. This situation contrasts sharply with that of THC, which mimics in many of its activities the endogenous cannabinoids. Cannabinoid receptors in the brain and the periphery bind THC but ignore CBD. Synthetic antagonists block THC (and endocannabinoid) action. None exist for the CBD effects. However, some recent observations may represent an opening toward elucidation of the CBD mechanism(s) of action.

### Stereospecificity of CBD Action

As indicated above, both (–) and (+) CBD are anticonvulsive. Also, both (–) and (+) CBD similarly suppress TNF production by LPS-activated mouse macrophages (unpublished observations). On this basis, it was assumed that the actions of CBD are nonstereospecific. Recent data show that this is not the case, at least as regards binding to the cannabinoid receptors.[16] While (+) CBD and most of the (+) CBD analogs bind to both CB1 and CB2 receptors, (–) CBD and its analogs are essentially inactive. Obviously, CBD does not act through the known cannabinoid receptors, but the stereospecificity observed may indicate action through some other biochemical system (e.g., binding to another type of receptor). The existence of numerous, not well-characterized, new cannabinoid receptors has been suggested.[81-86] Is CBD a ligand to one of these? Indeed, evidence has been brought forward that suggests that CBD is an antagonist of an as-yet-unidentified endothelial receptor for anandamide.[86]

### Inhibition of Anandamide Uptake

We have recently shown that CBD blocks anandamide uptake[16] and inhibits its enzymatic hydrolysis. If these effects are observed also in vivo, we may expect enhancement of endocannabinoid action, and at least

some of the CBD effects may in fact represent endocannabinoid actions.

### Antioxidative Effect

CBD, like many other cannabinoids, is a potent antioxidative agent.[45,87] CBD was more protective against glutamate neurotoxicity than either ascorbate or α-tocopherol. The neuroprotection exhibited by CBD was unaffected by cannabinoid receptor antagonists. In view of its liposolubility, it may exert (nonspecific?) action both in the periphery and in the brain as it crosses the blood-brain barrier.

## CONCLUSION

The nonpsychotropic CBD exhibits a plethora of effects, many of which may be of therapeutic importance or may serve as leads for pharmaceutical development.

It is unfortunate that the mechanism(s) of CBD action is still obscure; however, recent work on the stereospecificity of CBD action on its inhibition of anandamide uptake and hydrolysis, as well as on its antioxidative effects, may lead to elucidation of this longstanding enigma.

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# Amyotrophic lateral sclerosis

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**Amyotrophic lateral sclerosis (ALS) is an idiopathic, fatal neurodegenerative disease of the human motor system. In this Seminar, we summarise current concepts about the origin of the disease, what predisposes patients to develop the disorder, and discuss why all cases of ALS are not the same. In the 150 years since Charcot originally described ALS, painfully slow progress has been made towards answering these questions. We focus on what is known about ALS and where research is heading—from the small steps of extending longevity, improving therapies, undertaking clinical trials, and compiling population registries to the overarching goals of establishing the measures that guard against onset and finding the triggers for this neurodegenerative disorder.**

## Introduction

Since the 1990s, there has been growing scientific and clinical interest in amyotrophic lateral sclerosis (ALS). Advances in our understanding of the glutamate neurotransmitter system and the discovery of causal genes linked to the development of familial ALS have stimulated research interest, problems associated with clinical heterogeneity have been identified, and survival in ALS is now understood to be dependent on several factors, including clinical presentation (phenotype), rate of disease progression, early presence of respiratory failure, and the nutritional status of patients.

Extending life expectancy in ALS seems to be dependent on improving our understanding of its pathogenesis, which will lead to the development of early and specific diagnostic methods. There is a crucial need to formulate therapies that not only slow disease progression, but also deal with the secondary consequences of malnutrition and respiratory failure. At present, no definitive diagnostic test or biomarker for ALS exist, and neurologists rely on only clinical criteria for diagnosis. The development of novel biomarkers to objectively assess disease progression holds the promise of greatly refining therapeutic trial design and reducing trial costs. Furthermore, the power of population registries is being increasingly recognised as an essential adjunct to improved clinical assessment techniques. These collaborative endeavours will inevitably lead to a better understanding of ALS and its often unpredictable progression, and will lead to the development of guidelines for improved care of patients. In this Seminar, we provide an up-to-date overview of the key developments across the ALS specialty.

### Search strategy and selection criteria

We searched Medline (1966, to December, 2009), EmBase (1980, to December, 2009), and the Cochrane Library using the search terms "amyotrophic lateral sclerosis" or "motor neurone disease" in combination with "diagnosis", "epidemiology", "fronto-temporal dementia", "imaging", "neurophysiology", "management", and "neuroprotection". Further articles were included from reference lists, review articles, and major textbook chapters. Abstracts and reports from relevant meetings were also included. The final reference list was generated on the basis of originality and relevance to the topics covered in this Seminar. Emphasis was placed on publications from the past 5 years, but did not exclude commonly referenced and highly regarded older publications.

## Epidemiology and molecular genetics

Several factors have complicated epidemiological studies in ALS, including determination of a specific date of disease onset and the potentially long duration between onset of pathological changes and manifestation of clinical disease. This prodomal period between disease onset and presentation of symptoms possibly indicates the redundancy of neuronal populations. As a consequence, a range of epidemiological studies with rigorous designs and the use of unbiased patient cohorts have provided varying levels of evidence in support of different causative mechanisms of disease.[1,2] Population-based studies have established that the incidence of ALS in Europe is fairly uniform at 2·16 per 100 000 person-years.[3] Although ALS affects people worldwide, an exact incidence of this disease is not yet known.[4] Men have a higher incidence of disease (3·0 per 100 000 person-years; 95% CI 2·8–3·3) than do women (2·4 per 100 000 person-years; 95% CI 2·2–2·6), although the incidence between men and women is about the same in familial disease. The overall population-based lifetime risk of ALS is 1:400 for women and 1:350 for men. Peak age at onset is 58–63 years for sporadic disease and 47–52 years for familial disease. Incidence decreases rapidly after 80 years of age.[3]

Although the ALS phenotype might seem similar across populations, there are subtle differences in clinical presentation across European registries.[3] There is evidence from population-based studies that suggest that ALS is less common in individuals of mixed ancestral origin than in individuals of Spanish origin.[4] In a population-based mortality study from Cuba,[5] disease rates were 60% lower than in European and North American populations, lending support to previous observations of reduced frequency of ALS in those of Hispanic origin in North America.

About 5–10% of ALS is familial, with a Mendelian pattern of inheritance. To date, 13 genes and loci of major effect have been identified, many since 2009.[6,7] Of the known genes, mutations in *SOD1* (encodes for

copper/zinc ion-binding superoxide dismutase), *TARDBP* (also known as *TDP-43*; encodes for TAR DNA binding protein), *FUS* (encodes fusion in sarcoma), *ANG* (encodes angiogenin, ribonuclease, RNase A family, 5), and *OPTN* (encodes optineurin) cause a typical clinical phenotype. Mutations in *SOD1* induce a toxic gain of function, although the pathophysiology remains unclear. Both TDP-43[8] and FUS[9,10] (also known as TLS [translated in liposarcoma]) are multifunctional proteins involved in gene expression and regulation, including transcription, RNA splicing, transport, and translation. FUS and TDP-43 are also involved in the processing of small regulatory RNAs (microRNAs) and in RNA maturation and splicing. *ANG* is a hypoxia-responsive gene, which regulates RNA transcription.[11] *OPTN* is a causative gene of primary open-angle glaucoma. ALS-causing mutations of *OPTN* abolish the inhibition of activation of NFκB, and change the cytoplasmic distribution of optineurin.

Mutations in *SOD1* account for 20% of familial ALS[12] and 5% of apparently sporadic disease. Mutations in *TARDBP* account for 5–10% of familial ALS, mutations in *FUS* for 5%, and mutations in *ANG* for about 1%.

The remaining 90% of people diagnosed with ALS are classified as having sporadic disease. For these patients, results from family aggregation studies have identified an overlap between ALS and common neurodegenerative disorders, suggesting the existence of susceptibility genes that might increase the overall risk of neurodegeneration among relatives.[13] However, attempts to establish the complex genetic basis for sporadic ALS by identifying susceptibility genes have had little success. Results from candidate gene studies have identified several susceptibility genes,[7] although the relative contribution of every identified "at risk" gene rarely exceeds an odds ratio of 2·0, and the mechanism by which risk is conferred is not known.

Despite the disappointing findings in several recent genome-wide association studies of sporadic ALS,[4] a few possible genes have been identified. The main problem has been low power due to small sample sizes, with candidates being accordingly difficult to replicate in a second population.[7] The recent identification of two new susceptibility genes through collaborative research[14] suggests that further genes and pathways could be identified with increasingly effective cooperation between research groups. However, the poor track record of whole-genome association studies has led to a reconsideration of the "common disease, common variant" hypothesis in favour of a "common disease, multiple rare variant" hypothesis.

## Clinical phenotypes and prognosis

The varied presentations of ALS[15] are also crucial to the understanding and development of measures of disease progression.[16] The identification of specific phenotypes has important implications for patients, particularly with regards to prognosis and survival, but also for their enrolment in clinical trials.

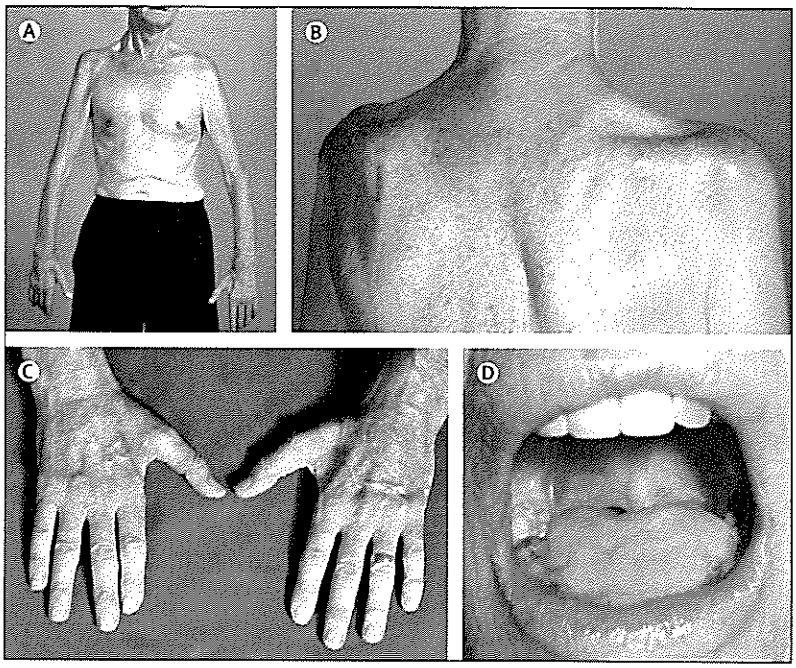


*Figure 1:* **Clinical features of muscles wasting in a patient with ALS**
Proximal and symmetrical upper limb wasting (A) results in an inability to lift arms against gravity ("man-in-the-barrel" or flail-arm variant ALS). Note the recessions above and below the scapular spine (B), indicating wasting of supraspinatus and infraspinatus muscles, as well as substantial loss of deltoid muscle. As a consequence, the glenohumeral joint becomes prominent, and prone to subluxation. (C) Disproportionate wasting of the thenar muscles combined with the first dorsal interossei, the so-called "split-hand", is a typical feature in ALS.[17] Although the mechanisms underlying this disproportionate wasting of hand muscles are unclear, a corticomotoneuronal origin has been proposed.[17] Specifically, the thenar muscles and first dorsal interossei receive more extensive corticospinal connections and thereby might be prone to glutamate-mediated excitotoxicity.[18] (D) Substantial wasting of the tongue muscles in bulbar-onset ALS. Note the absence of palatal elevation present on vocalisation. Difficulty with mouth opening and dysphagia might require supplementary feeding through a percutaneous endoscopic gastrostomy. In further support of a corticomotoneuronal hypothesis, the tongue is often disproportionately affected in comparison to other oropharyngeal musculature in patients with bulbar-onset ALS. As with the thenar muscles in the hand, the tongue receives more extensive cortical input than other muscle groups in the oropharyngeal area. ALS=amyotrophic lateral sclerosis.

The main presentations of ALS include: (1) limb-onset ALS with a combination of upper and lower motor neuron (UMN and LMN) signs in the limbs; (2) bulbar-onset ALS, presenting with speech and swallowing difficulties, and with limb features developing later in the course of the disease (figure 1); (3) the less common primary lateral sclerosis with pure UMN involvement; and (4) progressive muscular atrophy, with pure LMN involvement.[19]

The clinical hallmark of ALS is the presence of UMN and LMN features involving brainstem and multiple spinal cord regions of innervation. Patients can present with bulbar-onset disease (about 25%) or limb-onset disease (about 70%), or with initial trunk or respiratory involvement (5%), subsequently spreading to involve other regions.[20] Atypical modes of presentation can include weight loss, which is an indicator of a poor prognosis, cramps and fasciculations in the absence of muscle

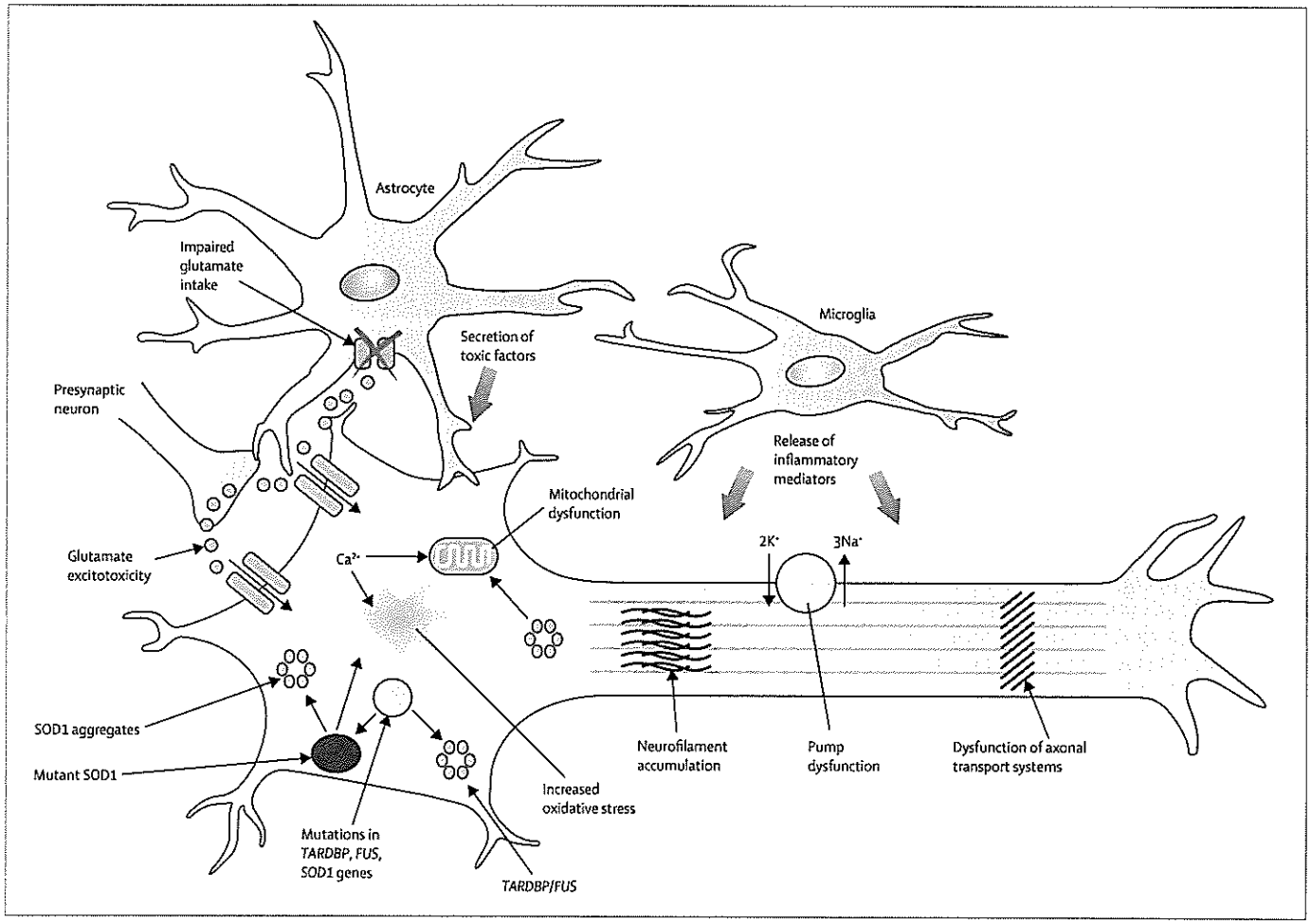


*Figure 2:* **Cellular and molecular processes mediating neurodegeneration in ALS**
The mechanisms underlying neurodegeneration in ALS are multifactorial and operate through inter-related molecular and genetic pathways. Specifically, neurodegeneration in ALS might result from a complex interaction of glutamate excitoxicity, generation of free radicals, cytoplasmic protein aggregates, SOD1 enzymes, combined with mitochondrial dysfunction, and disruption of axonal transport processes through accumulation of neurofilament intracellular aggregates. Mutations in *TARDBP* and *FUS* result in formation of intracellular aggregates, which are harmful to neurons. Activation of microglia results in secretion of proinflammatory cytokines, resulting in further toxicity. Ultimately, motor neuron degeneration occurs through activation of calcium-dependent enzymatic pathways. ALS=amyotrophic lateral sclerosis.

weakness, emotional lability, and frontal lobe-type cognitive dysfunction.[21]

In terms of presentation, UMN disturbance involving the limbs leads to spasticity, weakness, and brisk deep tendon reflexes. By contrast, LMN limb features include fasciculations, wasting, and weakness. Bulbar UMN dysfunction results in spastic dysarthria, which is characterised by slow, laboured, and distorted speech, often with a nasal quality.[22] The gag and jaw jerk can be pathologically brisk. Bulbar LMN dysfunction can be identified by tongue wasting, weakness, and fasciculations, accompanied by flaccid dysarthria and later dysphagia. Flaccid dysarthria results in nasal speech caused by palatal weakness, hoarseness, and a weak cough.[22]

ALS is relentlessly progressive—50% of patients die within 30 months of symptom onset and about 20% of patients survive between 5 years and 10 years after symptom onset.[23] Older age at symptom onset, early respiratory muscle dysfunction, and bulbar-onset disease are associated with reduced survival, whereas limb-onset disease, younger age at presentation, and longer diagnostic delay are independent predictors of prolonged survival.[23]

Some ALS subtypes tend to lead to a better prognosis. Specifically, flail-limb variant ALS (figure 1A, figure 1B) and progressive muscular atrophy, both predominantly LMN forms, are characterised by slower progression than other forms of ALS.[23,24] In the pure bulbar palsy phenotype, which typically affects women older than 65 years of age with disease remaining localised to oropharyngeal musculature and with UMN features predominating,[23] the prognosis varies from 2–4 years. Additionally, patients with primary lateral sclerosis progress more slowly than do patients with classic

ALS.[19,23] A definite diagnosis of primary lateral sclerosis should be delayed for at least 4 years from disease onset, given that development of LMN signs can occur even if the initial presentation appears that of a pure spastic syndrome.[25] Distinguishing these phenotypes from the typical ALS phenotype has implications for clinical trials of putative disease-modifying therapies.

Fatigue and reduced exercise capacity are common symptoms in ALS[26] and, ultimately, most patients need assistance with activities of daily living. Dysphagia develops in most patients with ALS, with consequent weight loss and malnutrition associated with poor prognosis.[27] Respiratory compromise eventually develops in most cases of ALS, leading to exertional dyspnoea, orthopnoea, hypoventilation with resultant hypercapnia, and early morning headaches.[28] Death becomes imminent once patients develop dyspnoea at rest. Progressive weakening of the respiratory muscles leads to respiratory failure, often precipitated by pneumonia.

## Overlap with frontotemporal dementia

The recent identification of TDP-43-positive ubiquitinated cytoplasmic inclusions in almost all cases of ALS, and more than half of patients with frontotemporal dementia (FTD), has rekindled interest in the overlap between these progressive neurodegenerative syndromes.[29] Although reported in early descriptions, overt cognitive symptoms and frank dementia were previously thought to be uncommon symptoms of ALS. Conversely, a few patients with FTD develop ALS.[30] Familial clustering of both disorders is also well recognised, with cases of FTD or ALS or coincident FTD-ALS presenting in families. The genes that cause these familial clusters are not yet known, but results from linkage studies have identified a common locus on chromosome 9.[31–35]

Cognitive deficits might initially have a subtle appearance and are often overlooked, but with appropriate cognitive and neuropsychological assessment, 20–50% of patients with ALS fulfil the consensus criteria for probable or definite FTD.[36] The most commonly encountered deficits involve executive function,[37] either affecting language or personality, with the cognitive profile most closely resembling that of behavioural-variant FTD. In terms of clinical implications, problems with judgment, impulsivity, and a general deterioration in the ability to undertake routine daily tasks can develop into difficult problems with management of patients.[38] Impaired verbal fluency, which is more prominent in patients with pseudobulbar disease, inevitably hinders the simple task of patients being able to communicate their needs. Cognitive, and particularly executive dysfunction, can also adversely affect patient compliance with treatment, decision-making abilities, and potentially raise ethical and medico-legal concerns.[37]

In further support of overlap between these two diseases, structural abnormalities, and specifically frontotemporal atrophy, have been identified by voxel-based morphometry MRI in patients with ALS and FTD-ALS. Bilateral atrophy of the motor and premotor cortices can develop,[30,39] although patients with FTD-ALS typically have more severe frontotemporal atrophy than do patients with ALS alone.[31,39] From a functional perspective, frontotemporal hypometabolism has been characterised in patients with ALS and FTD-ALS by use of 2-$^{18}$fluoro-2-deoxy-D-glucose PET.[33] This frontotemporal atrophy seems to be associated with neuronal loss and cortical gliosis on post-mortem pathology. As for most patients with sporadic ALS, intraneuronal inclusions (TDP-43-positive) are present in half of patients with FTD.[32,40] FUS-positive inclusions have been recently identified in patients with ubiquitin-positive, TDP-43-negative FTD and in patients with familial ALS caused by mutations in *FUS*[39,40]—further emphasising the pathological overlap between ALS and FTD.

*Panel 1:* Controversy in ALS—where does the disease begin?

- Despite Charcot's initial observation of concomitant UMN and LMN pathological changes in ALS, the question of where ALS begins has not been established. Resolution of this question might enhance the understanding of the pathophysiology of ALS and has diagnostic and therapeutic importance.
- The "dying-forward" hypothesis proposes that ALS is mainly a disorder of corticomotoneurons, which connect monosynaptically with anterior horn cells, mediating anterograde degeneration of anterior horn cells via glutamate excitotoxicity.
- Support for a dying-forward hypothesis includes:
  - Results from transcranial magnetic stimulation studies documenting that cortical hyperexcitability is an early feature in patients with sporadic ALS and precedes the clinical onset of familial ALS.
  - Clinical observations that: (1) motor neurons without a monosynaptic connection with corticomotoneurons, such as the oculomotor, abducens, and Onuf's nuclei, are typically spared in ALS; (2) the absence of a naturally occurring animal model of ALS is ascribed to a paucity of corticomotoneuronal-anterior horn cell connections; and (3) pure LMN forms of ALS are rare, whereas subclinical UMN involvement is invariably detected with transcranial magnetic stimulation studies.
- The "dying-back" hypothesis proposes that ALS begins within the muscle cells or at the neuromuscular junction. Specifically, there is deficiency of a motor neurotrophic hormone, which is normally released by postsynaptic cells and retrogradely transported up the presynaptic axon to the cell body where it exerts its effects.
- Support for the dying-back hypothesis includes:
  - Observations that synaptic denervation precedes the onset of motor neuron degeneration.
  - Synaptic denervation is mediated by accumulation of mutant SOD1 protein in Schwann cells.
- By contrast with the dying-forward and dying-back hypotheses, some investigators have proposed that UMN and LMN degeneration occur independently.

ALS=amyotrophic lateral sclerosis. LMN=lower motor neuron. UMN=upper motor neuron.

## Pathophysiological mechanisms

The pathophysiological mechanisms underlying the development of ALS seem multifactorial (figure 2), with emerging evidence of a complex interaction between genetic and molecular pathways.[41–43] ALS might be an

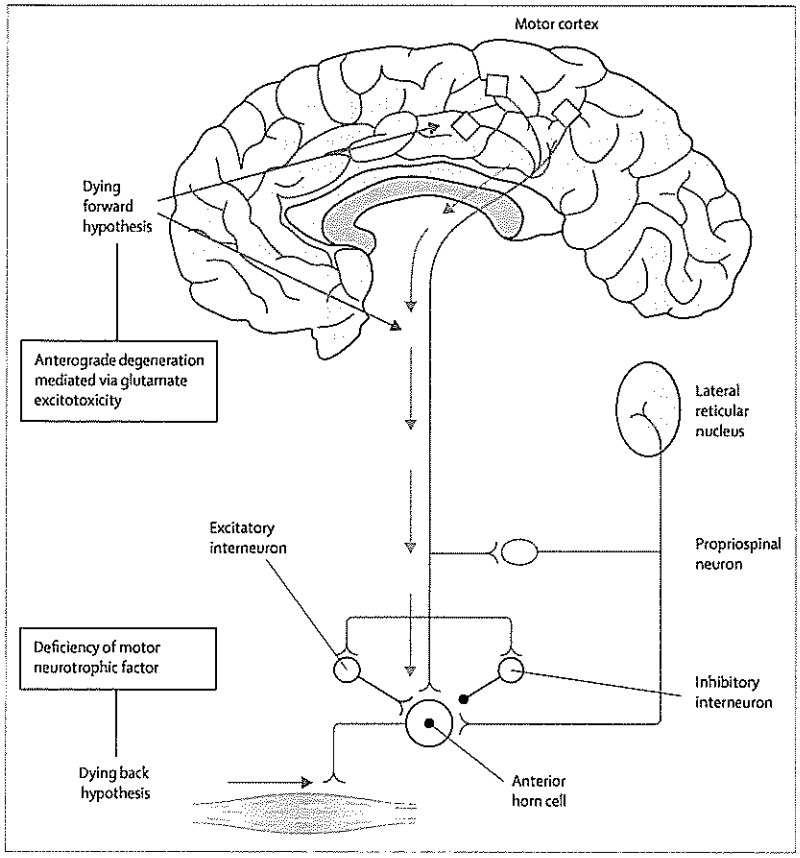


*Figure 3:* The "dying-forward" and "dying-back" hypotheses

adult manifestation of a developmental disorder of the human motor system. Specifically, in a Swedish case-control study,[1] low maternal age and high maternal age, and exposure to younger siblings, were associated with increased risk of developing ALS. Additionally, the development of the human motor system might potentially be perturbed during childhood by increased exposure to childhood infections, as occurs in families with young children. Various environmental risk factors for ALS have also been suggested, including a lifetime of intensive sport or physical exertion[44] and active service in the US armed forces.[45] In a retrospective study of football players from the Italian professional leagues, the standardised morbidity ratios were increased for development of ALS, particularly younger-onset disease.[46] For unknown reasons, footballers who played for more than 5 years, particularly in an active midfield position, were at highest risk of developing ALS. A cluster of ALS cases has also been reported in amateur football players from England.[47]

With regards to smoking, cigarettes might have a dose-dependent effect on the subsequent development of ALS.[48] Neurotoxins, including β-methyl-amino-L-alanine, were associated with the development of an epidemic of ALS-Parkinson's disease on the island of Guam.[49] This neurotoxic aminoacid was concentrated in the brains of patients with ALS-Parkinson's disease and entered the human food chain by consumption of flying foxes. These bats, a delicacy of native Guamanians, the Chamorro, feed on cycad seeds that have high concentrations of β-methyl-amino-L-alanine.[49]

No clear consensus has emerged to link *SOD1* mutations to the premature death of motor neurons. Current understanding links genetic mutations to a toxic gain of function of the SOD1 enzyme,[41] with generation of free radicals that eventually leads to cell injury and death.[50–53] Additionally, *SOD1* mutations induce conformational instability and misfolding of the SOD1 peptide, resulting in formation of intracellular aggregates[51,54] that inhibit normal proteosomic function, disrupting axonal transport systems and vital cellular functions.[50,51,55]

Glutamate-induced excitotoxicity has been implicated in ALS pathogenesis. Glutamate is the main excitatory neurotransmitter in the CNS, and binds to ionotropic N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors on the postsynaptic membrane.[56,57] Excessive activation of these postsynaptic receptors by glutamate, known as glutamate-induced excitotoxicity, can incite neurodegeneration through activation of calcium-dependent enzymatic pathways.[58,59] Glutamate-induced excitotoxicity can also result in generation of free radicals, which in turn can cause neurodegeneration by damaging intracellular organelles and upregulating proinflammatory mediators.[60,61]

The mechanism by which glutamate-induced excitotoxicity mediates motor neuron degeneration in human beings remains unclear (panel 1, figure 3). A so-called "dying-forward" process has been proposed, whereby UMN mediate anterograde degeneration of LMN by glutamate-induced excitotoxic processes.[62,63]

In addition to glutamate-induced excitotoxicity, structural abnormalities of mitochondria, dysfunction of the sodium/potassium ion pump, autophagy, and disrupted axonal transport systems have all been implicated in the pathogenesis of ALS.[64–70] Non-neuronal cells, such as astrocytes and microglia, might also directly contribute to neurodegeneration through mechanisms including insufficient release of neurotrophic factors, secretion of neurotoxic mediators, and modulation of glutamate receptor expression (known as non-cell autonomous neurodegeneration).[71]

Of further relevance, TDP-43 was recognised as a major component of ubiquitinated cytoplasmic protein aggregates in almost all patients with sporadic ALS, but not in the nucleus, as in normal neurons. Although there were questions about whether such aggregates triggered neurodegeneration in ALS, mutations in *TARDBP* were reported in 3% of familial ALS and 1·5% of patients with sporadic ALS, suggesting that TDP-43 aggregates have a central role in triggering ALS.[8,72] Evidence for the pathogenicity of

*TARDBP* mutations was suggested when mutations identified in highly conserved regions of DNA were not evident in controls, and segregated with the disease.[8,72] Given that TDP-43 binds both DNA and RNA, mutations in *TARDBP* could result in dysregulation of RNA processing.

Identification of *FUS* mutations on chromosome 16 associated with familial forms of ALS lends further support to this theory. FUS aggregates were not evident in patients with pathological changes in TDP-43 or SOD1, indicating a novel disease pathway.[10] Although the identification of a causative effect between mutations in the *TARDBP* and *FUS* genes and ALS was a major leap in understanding ALS pathogenesis, several factors need to be resolved. Do mutations in these DNA/RNA-binding proteins indicate a toxic gain or loss of function?[73] Does neurotoxicity result from the misfolded proteins overwhelming the cells' protein surveillance pathways or from sequestration of vital proteins and genomic material by TDP-43 and FUS aggregates? And what is the association between previously established pathophysiological mechanisms and the TDP-43 and FUS proteins?

## Diagnosis

Without a diagnostic test for ALS, clinicians mostly rely on identifying the combination of UMN and LMN signs in the same body region, with subsequent evidence of disease progression to other regions. The El Escorial criteria,[74] revised in 1997,[75] use a combination of UMN and LMN signs to establish levels of diagnostic certainty. Clinical trial investigators have tended to enrol patients with either probable or definite ALS according to the El Escorial criteria, highlighting their universality, although inclusion of these diagnostic features as enrolment criteria might be argued as restrictive.[76] Furthermore, these criteria can have poor sensitivity, particularly in the early stages of ALS when patients are most likely to benefit from therapeutic intervention.[77] Because of these criticisms, the criteria have been modified to help early diagnosis[78] and to optimise levels of diagnostic certainty, important in the clinical trial setting.[79]

There is often a long delay before a definitive diagnosis is reached, partly because of the insidious onset of symptoms, with the median time to diagnosis of about 14 months.[80] Unusual clinical presentations, a low index of suspicion, and misinterpretation of neurophysiological or neuroradiological findings are common causes of diagnostic uncertainty.[15] Unfortunately, diagnostic delay can lead to use of inappropriate therapies, a delay in starting appropriate pharmacological and symptomatic therapies, and problems in dealing with psychosocial factors.

The diagnosis of ALS is devastating for the patient and family members, and must be handled sensitively. Patients and family members can carry the emotional burden of an insensitively delivered diagnosis for the entire disease course, and initial indecision about the diagnosis in atypical cases can delay the process of accepting the terminal prognosis of the disease. Scheduling a follow-up appointment soon after diagnosis is beneficial to answer questions not dealt with during the initial consultation and can help provide further information about support networks, which are well established in most developed nations.[81]

Although rare, the existence of several disorders that mimic ALS necessitates a thorough diagnostic

*Panel 2:* Differential diagnosis of ALS and appropriate investigations

**Disorders of motor neurons**
- Spinal muscular atrophy (*SMN* gene deletion assay)
- X-linked spinobulbar muscular atrophy (Kennedy's disease; increased CAG repeats in DNA from blood)
- Poliomyelitis or post-polio syndrome (history, NCS, electromyography)
- Hexosaminidase A deficiency (white-cell enzyme testing)

**Disorders of motor nerves**
- Multifocal motor neuropathy (NCS, electromyography, ganglioside GM1 antibodies)
- Chronic inflammatory demyelinating neuropathy (NCS, lumbar puncture)
- Cramp-fasciculation syndrome (NCS, electromyography)
- Neuromyotonia (antibodies to voltage-gated potassium channels)
- Hereditary spastic paraparesis plus (gene mutation testing)
- Hereditary motor neuropathy with pyramidal features
- Radiculoplexopathy (NCS, electromyography, MRI)
- Paraneoplastic syndrome (serum markers, imaging, bone marrow biopsy sample)
- Heavy metal poisoning (urine or blood screens)
- Mononeuritis multiplex (NCS, electromyography, vasculitic screen, serology)

**Disorders of neuromuscular junction**
- Myasthenia gravis (acetylcholine receptor antibodies, MuSK antibodies, repetitive stimulation, single-fibre electromyography)
- Lambert-Eaton myasthenic syndrome (repetitive stimulation)

**Structural CNS and spinal lesions**
- Syringomyelia or syringobulbia (MRI)
- Tabes dorsalis (syphilis serology)
- Multiple sclerosis (MRI, oligoclonal bands, evoked responses)
- Monomelic spinal muscular atrophy (Hirayama's disease; electromyography, MRI)
- Lyme disease (Lyme serology)
- Human T-lymphotropic virus-1 (HIV)

**Myopathy**
- Inclusion body myositis (electromyography, CK, muscle biopsy sample)
- Polymyositis (electromyography, CK, muscle biopsy sample, autoimmune screens)
- Dermatomyositis (electromyography, CK, skin, and muscle biopsy sample)
- Polyglucosan body disease (NCS, electromyography, muscle or nerve biopsy sample)

**Endocrine**
- Thyrotoxicosis (thyroid function tests, electromyography, muscle biopsy sample)
- Hyperparathyroidism (calcium ion and parathyroid testing)
- Subacute combined degeneration (vitamin $B_{12}$ concentrations)
- Coeliac disease (serum testing, bowel biopsy sample)

ALS=amyotrophic lateral sclerosis. CK=creatine kinase. NCS=nerve conduction studies. MuSK=muscle-specific tyrosine kinase.

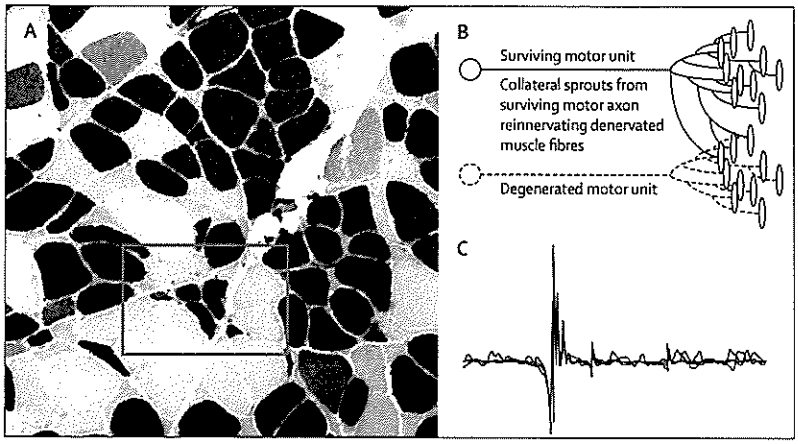


*Figure 4:* **Investigation findings in ALS**
(A) Biopsy sample of the left vastus lateralis muscle from a patient with ALS, stained with ATPase pH 9·4. The biopsy sample highlights grouped atrophic fibres with both type I and type II fibres (mixed-type fibres, encompassed by red box). (B) Pathophysiology of motor unit degeneration and reinnervation; with superimposition (C) of ten traces demonstrating the typically large, polyphasic, unstable (complex) motor units observed in established ALS (sweep duration 50 ms), with late components, indicating some re-innervation. ALS=amyotrophic lateral sclerosis.

assessment, which usually includes structural imaging and neurophysiological and laboratory investigations, to reduce the likelihood of an incorrect diagnosis (panel 2).[20] In cases of pure LMN syndromes, genetic testing for Kennedy's disease, an X-linked bulbospinal atrophy, and spinal muscular atrophy is important.[82] Muscle biopsy samples can be of further diagnostic value for excluding unusual myopathies such as polyglucosan body disease[83] or for confirming the presence of ALS by indicating atrophy of mixed-fibre types (figure 4A).[84]

Routine neurophysiological investigations of patients with ALS include nerve conduction studies, electromyography, and, less commonly, transcranial magnetic stimulation.[20,85] Nerve conduction studies are essential to exclude disorders that mimic ALS, particularly demyelinating motor neuropathies.[86] Motor nerve conduction is normal in the early stages of ALS, but in advanced disease the compound muscle action potential amplitude becomes reduced, indicating denervation.[87] Sensory nerve conduction is typically normal in patients with ALS, differentiating ALS from demyelinating neuropathies.[88] Prominent abnormalities of sensory nerve conduction studies should raise suspicion of an alternative diagnosis. In patients presenting with predominantly LMN findings, treatable disorders such as multifocal motor neuropathy should be taken into account, with indication of conduction block in at least two motor nerves outside the common entrapment sites.[89]

In addition to nerve conduction studies, electromyography is useful for the identification of LMN loss (figure 4B). The electromyographical findings indicating LMN loss include fibrillation potentials, positive sharp waves, and chronic neurogenic changes (figure 4C).[86,90] These electromyographical abnormalities have been recently incorporated into the revised El Escorial criteria to help with the diagnosis of ALS, complementing the clinical features of LMN involvement.[90] Fibrillation potentials and positive sharp waves can be evident in muscles that seem clinically normal.[86] Electromyography can therefore help with an early diagnosis by establishing the presence of subclinical LMN involvement.

Motor units that survive can fire spontaneously as fasciculation potentials, clinically visible as involuntary muscle twitching—a typical feature of ALS.[91] When detected in the tongue, fasciculations are highly specific for ALS.[92] The presence of fasciculations in the absence of other electromyographical findings should be interpreted with caution and can be a sign of less serious disorders, especially "benign" cramp-fasciculation syndrome.[93] Conversely, recently revised consensus guidelines (known as the Awaji Island criteria) have recommended that fasciculations should be thought to be equivalent to fibrillation potentials in individuals with clinically suspected ALS.[90] Furthermore, fasciculations in ALS are complex ("malignant"), indicating re-innervation, and have diagnostic importance when combined with chronic neurogenic changes (figure 4C).

There is discussion about how successful clinicians are at diagnosing ALS when using the combined approaches of clinical assessment and laboratory investigation. This matter was taken into account by the Scottish ALS registry, which identified a false positive rate of 8%.[15] Other data from population-based studies have reported similar false-positive rates, with a false-negative rate approaching 44%.[94,95] In false-positive cases, the main reasons for diagnostic revision included failure to progress, development of atypical features, and results of follow-up neurophysiological and neuroradiological investigations.[15,94] Multifocal motor neuropathy was the most frequent disorder misdiagnosed as ALS, followed by Kennedy's disease.[94]

## Advances in neuroimaging

The greatest contribution of neuroimaging to the diagnostic pathway in ALS so far has been the ability of MRI to exclude alternative pathological causes. However, the imaging discipline is evolving, and multimodal neuroimaging has made major progress in the confirmation that ALS is a multisystem cerebral neurodegenerative disorder.[96] The key neuroimaging findings, some with potential as biomarkers, are discussed in panel 3.

## Management and prevention

Riluzole, an inhibitor of glutamate release, is a disease-modifying (neuroprotective) therapy for patients with ALS (panel 4). In two large randomised controlled trials, riluzole extended survival of patients by 3–6 months.[126–128] This benefit seemed greater for management of patients in specialised multidisciplinary ALS clinics than in other settings,[129] with most beneficial effects seen in patients with moderate functional impairment.[130]

*Panel 3:* Key neuroimaging findings in ALS

**MRI corticospinal tract hyperintensity**

Hyperintensity of the corticospinal tracts as seen on MRI can be prominent in ALS,[85,97] but this feature is not specific to the disease (figure 5A).

**Cerebral atrophy detection with MRI**

Voxel-based morphometry has quantified grey and white matter to detect cerebral atrophy in patients with ALS[98] linked to cognitive impairment,[99] with notable differences in regional emphasis between patients with sporadic disease and those with familial disease who have a longer life expectancy.[100] 3-Dimensional rendering of the brain by use of MRI might also serve to highlight focal abnormality (figure 6).

**Magnetic resonance spectroscopy**

The measurement of proton-containing metabolites such as *N*-acetylaspartate (expressed as a ratio with creatine/phosphocreatine or choline) has served as a marker of neuronal loss. Patients with ALS have a reduced primary motor cortex *N*-acetylaspartate to creatine ratio compared with controls,[101] and use of magnetic resonance spectroscopy seems particularly sensitive in the detection of upper motor neuron dysfunction, distinguishing patients with progressive muscular atrophy from those with ALS.[102]

**Diffusion tensor imaging**

Diffusion tensor imaging can be used to exploit the sensitivity of MRI to identify the direction of water diffusion, which is expected to be restricted (ie, anisotropic) within intact neuronal pathways and more diffuse (isotropic) in regions of reduced integrity. Quantifiable measures such as fractional anisotropy and mean diffusivity are powerful surrogate markers of neuronal pathological changes,[103] and inter-connectivity between neuronal pathways can be mapped using the allied technique of tractography (figure 5B).[104] Use of diffusion tensor imaging can detect reduced fractional anisotropy within the corticospinal tract of patients with ALS.[105]

**Functional studies**

Results of PET activation studies with 2-$^{18}$fluoro-2-deoxy-D-glucose and $H_2{}^{15}O$ have indicated widespread extramotor changes in patients with ALS,[106] with frontal deficits linked to neuropsychological impairment,[107] providing clear application to the emerging clinicopathological overlap between ALS and FTD.[108] Non-invasive study of brain activation by functional MRI exploits differences in the resonant properties of oxyhaemoglobin versus deoxyhaemoglobin (blood oxygenation level dependent [BOLD]-functional MRI). By analysing whole-brain BOLD-functional MRI activity in the resting state, functionally interconnected brain regions can be identified.[109] Results from studies in patients with ALS have shown both "default mode" and sensorimotor network activation changes.[110] This technique has the potential to further delineate the extramotor cerebral pathological changes in patients with ALS.

**Molecular imaging**

Receptor ligand PET has been used to study molecular mechanisms in ALS. Data from $^{11}$C-flumazenil PET have indicated reduced inhibitory GABAergic cortical effects in ALS,[111] in keeping with the hypothesis of cortical hyperexcitability as a fundamental aspect of ALS pathogenesis.[112] Use of the benzodiazepine receptor PET ligand $^{11}$C-PK11195 revealed widespread microglial activation in ALS,[113] supported by the finding of inflammatory biomarkers in the cerebrospinal fluid.[114] The pronounced frontotemporal reductions in the binding of the 5-HT1A receptor ligand $^{11}$C-WAY100635 in patients with ALS,[115] and data from neuropathological receptor studies that revealed similar changes in FTD,[116] suggest that serotonergic mechanisms warrant further study in relation to pathogenesis. Finally, paramagnetic properties of small particles of iron oxide, which can be used as intravenous contrast agents, might indicate the start of the era of molecular MRI,[117] with potential to understand inflammatory mechanisms[118] and therapeutic stem-cell tracking.[119]

**Detection of presymptomatic markers of disease**

The poor definition of the population at risk for sporadic ALS impedes attempts to identify an early, presymptomatic diagnostic biomarker. Results from a diffusion tensor imaging study of presymptomatic patients with a highly penetrant dominant *SOD1* gene mutation revealed changes in the posterior limb of the internal capsule not seen in healthy controls, which might be among the earliest detectable changes.[120]

ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia.

Symptomatic treatments remain the cornerstone of management for patients with ALS (panel 5).[28] For some patients, these treatments not only alleviate symptoms but also improve survival and quality of life.[131] Optimum care for patients with ALS is provided within a multidisciplinary environment where physiotherapists, occupational therapists, speech therapists, respiratory physicians, gastroenterologists, and social workers collaborate to guide symptomatic management through the course of disease.[132] Multidisciplinary models of care have developed as a predictor of survival, reducing the risk of death by 45% at 5 years. Compared with patients managed in a general neurology clinic, patients managed in a specialised clinic had a better quality of life, possibly attributable to more effective use of resources, with benefits derived after a single visit.[132]

Respiratory function and nutrition are crucial symptomatic concerns for patients with ALS, with respiratory failure being the main cause of death.[81] Expert consensus guideline recommendations have been developed for key care concerns in ALS, including respiratory management, nutrition, and palliative care.[81,131] A positive outlook should be emphasised.[133]

*Panel 4:* Controversy in ALS—clinical trials

Although clinical trials of ALS have been done since the 1980s, riluzole is the only drug of proven efficacy for treatment of this disorder. The failure of ALS clinical trials to lead to substantial benefits has been attributed to several potential design problems at preclinical and clinical levels:

**Preclinical**

- Inappropriate mouse model. Until recently, the SOD1 mouse model has been the benchmark for testing potential neuroprotectants in ALS.[121] However, as *SOD1* mutations account for about 2% of all ALS cases, the mouse model might have little relevance to human sporadic disease. Furthermore, this model undergoes a series of stereotypical changes that begin with hind limb weakness. The recent development of mouse models with mutations in the gene encoding TDP-43 is a potential advance in therapeutic development for ALS, providing basic scientists with a new, perhaps more relevant, platform for studying novel therapies.[122]
- Inappropriate timing of introducing drugs and dosing problems. Some investigators have studied the effects of presymptomatic delivery of drugs on disease onset.[123] Although this timing might contribute towards understanding the subclinical processes that underlie motor neuron degeneration, it seems to be of little relevance for treatment of sporadic ALS. Many preclinical studies have also examined ultra-high doses of drugs that would probably translate into plasma concentrations far beyond that tolerable by human patients. Some investigators have advocated that the highest tolerable dose should not be assumed to produce the best outcome.[124]

**Clinical**

- Trial design. There is increasing need for more effective screening of pharmacological drugs during phase 2 trials because after this period of clinical development a decision is made to proceed with confirmatory testing (ie, a phase 3 trial) or to reject the drug as ineffective. The distinction between phase 2 and phase 3 trials in ALS becomes blurred, because providing preliminary evidence of drug efficacy in this disease at the phase 2 level is difficult. The absence of an effective biomarker is a major contributing reason. Therefore, the dilemma remains whether to use efficient statistical strategies for minimising trial duration and sample size, to increase the chance of proceeding with a phase 3 trial (ie, higher false-positive rate), or to do phase 3 clinical trials tailored as phase 2 clinical trials (ie, higher false-negative rate). The latter approach is perhaps one that is now rarely done, given recent advances in statistical design.[125] Investigators are also proceeding with phase 3 clinical trials, even in the absence of preliminary evidence of efficacy in human patients.
- Choice of primary endpoint. Changes in the primary endpoint establish whether a trial will be successful. The choice of the correct primary endpoint in ALS clinical trials has been debated. Trials that use functional scales and measures of strength as primary endpoints have dominated the field, whereas trials mainly concerned with improved survival of patients have been few and far between recently. The design and clinical benefits of the former include: smaller sample size, shorter trial duration, and clinically meaningful treatment effects. Nevertheless, measurement of survival might be the only means of determining whether a treatment effect truly exists, given the large extent of motor neuron loss from the time of symptom onset. For example, riluzole has small but significant effects on the function of patients, detectable with sample sizes far beyond that required to realise its survival benefit.

ALS=amyotrophic lateral sclerosis.

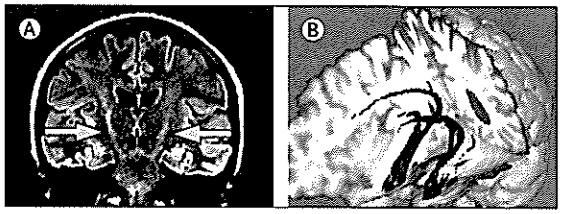


*Figure 5:* **Standard and experimental MRI sequences in patients with ALS**
(A) T2-weighted FLAIR sequence shows hyperintense corticospinal tracts in a patient with ALS on this coronal view (arrows), but this feature is neither sensitive nor specific in the absence of other more obvious clinical symptoms. (B) Diffusion tensor tractography is a research-based MRI technique that has potential to study extramotor and motor neuronal pathway involvement in ALS (superior oblique cut-out brain section viewed from left). In this patient with an unusual ALS phenotype that included prominent aphasia, reconstruction of the temporal lobe white matter projection fibres indicated that there were fewer fibres on the left (blue) compared with the right (red) side. ALS=amyotrophic lateral sclerosis. FLAIR=Fluid-attenuated inversion-recovery.

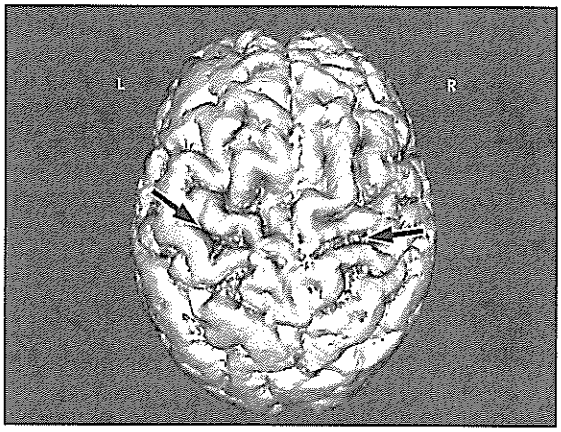


*Figure 6:* **3-Dimensional MRI of a brain of a patient with primary lateral sclerosis, as shown from above**
Arrows show visibly widened precentral sulci with relative atrophy of the adjacent gyri, notably the motor strips. Macroscopic atrophy as seen here is rare in patients with typical ALS, but is more frequently noted in those with primary lateral sclerosis, who have a predominantly upper motor neuron burden of disease. This figure highlights the late stage of a corticomotoneuronal process postulated to be inherent in ALS more generally. ALS=amyotrophic lateral sclerosis.

Respiratory failure indicates combined degeneration of central respiratory centres and motor neurons contributing to the phrenic nerve. Respiratory compromise is commonly present at diagnosis in patients with ALS.[28] Nocturnal hypoxia, and associated symptoms of lethargy, loss of concentration, morning headaches, and unrefreshed sleep are consequences of central dysfunction. Diaphragmatic weakness can be diagnosed with spirometry, with vital capacity undergoing a progressive decline over the course of disease. Measures of inspiratory muscle strength, such as maximum inspiratory pressure and sniff nasal inspiratory pressure, are more accurate predictors of respiratory dysfunction than is vital capacity, and might be more feasible in patients with substantial facial muscle weakness (ie, who are unable to form a tight lip seal).[28,81]

Although undertaking polysomnography might be the optimum approach to identify nocturnal hypoxic episodes, nocturnal pulse oximetry is usually adequate in patients with ALS.[81] Non-invasive ventilation improves quality of life in patients with ALS and improves survival.[134] Guidelines for instituting non-invasive ventilation rely on a combination of symptoms that

*Panel 5:* Symptomatic care in ALS

**Weakness and disability**
- Orthotics (eg, ankle foot orthosis, neck collars)
- Physiotherapy
- Adaptive aids (eg, walking frame, wheelchair)

**Dysphagia**
- Assessment by speech therapist and dietitian
- Safe swallowing techniques and modified diet
- Insertion of gastrostomy tube

**Dyspnoea and poor cough**
- Ventilatory support
- Morphine or benzodiazepines
- Chest physiotherapy
- Suction machine
- Manually assisted coughing techniques

**Pain (ie, musculoskeletal pain and cramps, fasciculations and spasticity, skin pressure pain caused by immobility)**
- Physiotherapy, NSAIDs
- Muscle relaxants (baclofen, botulinum toxin)
- Anticonvulsants (eg, gabapentin)
- Re-positioning and pressure area care
- Opioid drugs
- Pressure-relieving cushions and mattress

**Dysarthria**
- Assessment by speech pathologist
- Communication aids
- Educate family and caregivers

**Cognitive changes (frontal lobe dysfunction or dementia)**
- Explain symptomatology to caregivers and family
- Antidepressant therapies

**Sialorrhoea**
- Anticholinergic antidepressants (eg, amitriptyline)
- Anticholinergic drugs (eg, glycopyrronium bromide)
- Botulinum toxin injections
- Radiation of salivary glands
- Mouth-care products
- Suction

**Thickened saliva**
- Natural remedies (eg, papaya)
- Ensure adequate hydration
- Saline nebulisers; nebulised *N*-acetylcysteine
- Suctioning of the mouth
- Mouth care

**Emotional lability**
- Educate patients with ALS and caregivers
- Amitriptyline
- Benzodiazepines
- Dextromethorphan hydrobromide/quinidine sulfate

**Depression and anxiety**
- Counselling
- Benzodiazepines
- Antidepressants

**Sleep disturbance**
- Treat underlying problem
- Respiratory review, non-invasive ventilation
- Benzodiazepines, tricyclic antidepressants

**Constipation**
- Dietary changes (eg, increase fluid and fibre intake)
- Use formulations high in bran, bulk, or fibre
- Regular oral aperients (Movicol [Norgine, the Netherlands] or suppositories).

ALS=amyotrophic lateral sclerosis. Data from Andersen and colleagues[81] and Miller and colleagues.[121]

signify respiratory muscle weakness (dyspnoea and orthopnoea), along with signs of respiratory muscles weakness, including substantial desaturation on overnight oximetry, increased partial pressure of carbon dioxide ($PCO_2$) of less than 65 mm Hg and reduced forced vital capacity of less than 80% or sniff nasal inspiratory pressure of less than 40 $cmH_2O$.[28,81] Patients with substantial bulbar impairment and sialorrhoea might not tolerate non-invasive ventilation, and appropriate management of secretions is crucial.[28] In patients with ALS who are intolerant of non-invasive ventilation, when this form of ventilation is no longer sufficient because of progressive respiratory muscle weakness, invasive ventilation via tracheostomy is an option.[81] Although invasive ventilation prolongs survival, this approach is rarely used in most countries because of the practical challenges involved, the expense, and the profound loss of quality of life.[135] In terms of symptomatic therapy, subcutaneous morphine provides great relief in patients who have dyspnoea at rest.[81,136]

Malnutrition is a key determinant of prognosis.[137] The development of malnutrition in ALS is multifactorial, and includes reduced food intake secondary to dysphagia, as well as hypermetabolism.[81,138] About 50–60% of patients with ALS have a hypermetabolic state,[139,140] which seems to be stable over the course of the disease and is dependent on age, sex, and fat-free mass.[139] The increase in metabolic rate, as measured by resting energy

*Panel 6:* Controversy in ALS—alternative and off-label treatments

Given the terminal nature of ALS, the fact that patients are often willing to experiment with unproven therapies is not surprising. Popular alternative and off-label treatments have included insulin-life growth factor-1, lithium carbonate,[70] minocycline,[144] and stem-cell therapy. Patients should take caution when starting alternative and off-label treatments. As identified by some ALS clinical trials, some treatments can accelerate the progression of muscle weakness and negatively affect survival.

To keep the ALS community informed of available alternative and off-label treatments, an internet-based initiative, ALSUntangled, has been established recently.[145] ALSUntangled enables the exchange of information about new alternative and off-label treatments between patients with ALS and clinicians. Patients with ALS are encouraged to share newly hypothesised alternative and off-label treatments, as the goal of this initiative is to consolidate and convey information about cost, scientific and ethical basis, and potential benefits and risks of every so-called treatment.

For more on ALSUntangled see http://www.alsuntangled.com/index.html

ALS=amyotrophic lateral sclerosis.

expenditure, is associated with reduced survival.[139] Although the mechanisms that underlie a hypermetabolic state are unclear, dysfunction of muscle mitochondria is implicated in the pathogenesis of ALS.[141]

Insertion of a percutaneous gastrostomy tube ensures sufficient caloric and fluid intake, and should be offered to patients who have substantial weight loss, even in the absence of dysphagia.[81] Implementing a gastrostomy should be discussed early in the disease course because morbidity increases when vital capacity is less than 50%.[142]

Attention to the many symptoms that might develop during the course of the disease is essential to improve the quality of life for patients with ALS (panel 5).[143] The terminal phase of ALS can be associated with restlessness, anxiety, pain, and dyspnoea, and well coordinated multidisciplinary palliative care is needed. Finally, patients might also seek alternative treatments (panel 6), often with little evidence of benefit for ALS, and at great personal financial cost.[146]

## Conclusions

> "Let us keep looking, in spite of everything. Let us keep searching. It is indeed the best method of finding, and perhaps thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same we must give this man today."
>
> *Charcot (1889)*

By contrast with the previous century of little progress, the recent developments in understanding, particularly with regards to the genetics, clinical phenotypes, and more general pathophysiology of ALS, encourage realistic hope that new treatment approaches will emerge.

**Contributors**

BCC and MCZ did the literature search and contributed to sections on management and prevention of ALS. OH and MRT contributed to the review of the literature and the writing of the paper. MCK did the literature review, coordinated authors' writing, revision, and editing, wrote the first draft, prepared figures, and finalised the manuscript. JRB wrote the section on clinical phenotypes and on FTD-ALS, and was involved in revising the manuscript. SV searched the literature, was involved in writing and proofreading the paper, and helped prepare the figures. AE contributed to the sections on management and prevention of ALS and to the writing of the paper.

**Conflicts of interest**

OH has consulted for ONO Pharmaceuticals and KNOPP Pharmaceuticals, and has received research support from Sanofi-Aventis and Serono Pharmaceuticals. OH has received advisory board fees from Novartis, Biogen, and Merck Sorono, and has received travel and accommodation sponsorship from Merck Sorono. She is the inventor of a patent held by the Royal College of Surgeons in Ireland for the use of angiogenin as a therapeutic in ALS. Funding sources include the National Health and Medical Research Council of Australia (project grants 510233 and 568743; MCK); the Motor Neurone Disease Research Institute of Australia (MCK); the Irish Health Research Board (OH); American ALS Association (OH); the Irish Motor Neurone Disease Research Foundation (OH); and the Medical Research Council (Lady Edith Wolfson Clinician Scientist Fellowship; MRT). SV has received the Clive and Vera Ramacciotti grant and the Charles Viertel grant, and has received fees for advisory board from Merck Serono Australia, Novartis Australia, and Biogen (not related to the topic covered in this paper). BCC, AE, JRB, and MCZ declare that they have no conflicts of interest.

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# Cannabis and Amyotrophic Lateral Sclerosis: Hypothetical and Practical Applications, and a Call for Clinical Trials

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**Abstract**

Significant advances have increased our understanding of the molecular mechanisms of amyotrophic lateral sclerosis (ALS), yet this has not translated into any greatly effective therapies. It appears that a number of abnormal physiological processes occur simultaneously in this devastating disease. Ideally, a multidrug regimen, including glutamate antagonists, antioxidants, a centrally acting anti-inflammatory agent, microglial cell modulators (including tumor necrosis factor alpha [TNF-α] inhibitors), an antiapoptotic agent, 1 or more neurotrophic growth factors, and a mitochondrial function-enhancing agent would be required to comprehensively address the known pathophysiology of ALS. Remarkably, cannabis appears to have activity in all of those areas. Preclinical data indicate that cannabis has powerful antioxidative, anti-inflammatory, and neuroprotective effects. In the G93A-SOD1 ALS mouse, this has translated to prolonged neuronal cell survival, delayed onset, and slower progression of the disease. Cannabis also has properties applicable to symptom management of ALS, including analgesia, muscle relaxation, bronchodilation, saliva reduction, appetite stimulation, and sleep induction. With respect to the treatment of ALS, from both a disease modifying and symptom management viewpoint, clinical trials with cannabis are the next logical step. Based on the currently available scientific data, it is reasonable to think that cannabis might significantly slow the progression of ALS, potentially extending life expectancy and substantially reducing the overall burden of the disease.

**Keywords**

cannabis, endocannabinoids, amyotrophic lateral sclerosis, clinical trials, motor neuron disease

## Introduction

Amyotrophic lateral sclerosis (ALS), with an incident rate of 5 to 7 per 100 000 population, is the most common form of adult motor neuron disease.[1] Amyotrophic lateral sclerosis is a rapidly progressive neuromuscular disease that destroys both upper and lower motor neurons, resulting in weakness, spasticity, and ultimately death from respiratory failure. The vast majority of ALS cases are acquired and occur sporadically. Emerging evidence suggests that increased oxidative stress from free radical toxicity and/or excessive glutamate activity is what leads to motor neuron cell death in the brain and spinal cord.[2-5] Inherited forms of the disease, which occur in approximately 5% to 10% of all patients with ALS, are largely because of mutations in the superoxide dismutase gene, presumably producing a marked increase in oxidative stress. Presentations of familial ALS have more variability than in sporadic ALS and are mutation specific with the most aggressive form because of the A4V mutation.[5] Recent results have established that ALS also involves other nonneuronal cells including astroglia and microglia.[6,7] Other putative mechanisms involved in motor neuron degeneration in ALS include mitochondrial dysfunction, neuroinflammation, growth factor deficiency, and glutamate excitotoxicity.[2,3]

Significant advances have been made regarding our understanding of the molecular mechanisms of ALS.[8-12] However, this has not yet translated into an effective therapeutic treatments. To date, the only food and drug administration- (FDA) approved therapy available for ALS is the antiglutamatergic agent Riluzole, which has limited therapeutic efficacy.[10] Given

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this perspective, there remains an ongoing search for novel therapeutic approaches. There is increasing evidence that cannabinoids and manipulation of the endocannabinoid system may have beneficial disease-modifying potential in ALS.[13-21] Moreover, the clinical effects of cannabis, the principal cannabinoid-producing botanical agent, have been reported to be useful in managing the symptomatology in ALS, as well as many other neurodegenerative disorders.[22-34] Thus, significant efforts are now being directed at evaluating the role of the endocannabinoid system in the pathophysiology of ALS. In addition, there is an emerging body of science that points to a role of exogenous cannabinoids in both clinical symptom management and a positive disease-modifying effect.[13-21]

## The Physiology and Pharmacology of Cannabinoids

Prior to the last decade, there was little known about the specific pharmacological and molecular effects of cannabis. However, important advances have taken place recently, which have greatly increased the understanding of the receptors and ligands composing the endogenous cannabinoid system.[35-54] Research has shown that 2 major cannabinoid receptor subtypes exist, including the cannabinoid receptor, type 1 (CB1) subtype, which is predominantly expressed in the brain, and the cannabinoid receptor, type 2 (CB2) subtype, which is primarily found on the cells of the immune system.[35,49,50] A variety of ligands for these receptors based on the cannabinoid structure have been synthesized and studied. Experiments performed with several types of neural cells that endogenously express the CB1 receptor suggest that activation of protein kinases may be responsible for some of the cellular responses elicited by these receptors.[51] The discovery of the endocannabinoids, that is, endogenous metabolites capable of activating the cannabinoid receptors, and the understanding of the molecular mechanisms leading to their biosynthesis, release, and inactivation, have created a new area in research on the pharmaceutical applications of cannabinoid-based medicines.[52] The characterization of endocannabinoids such as anandamide and the detection of widespread cannabinoid receptors in the brain and peripheral tissues suggest that the cannabinoid system represents a previously unrecognized ubiquitous network in the nervous system.

Cannabinoid receptors are G protein-coupled, 7-segment transmembrane proteins, similar to the receptors of other neurotransmitters such as dopamine, serotonin, and norepinephrine.[51,52] Dense receptor concentrations are found in the cerebellum, basal ganglia, and hippocampus, likely accounting for the effect of exogenously administered cannabinoids on motor tone and coordination as well as mood state.[53-55] Low concentrations are found in the brain stem, accounting for the low potential for lethal overdose with cannabinoid-based medicines.[56-59] A growing number of strategies for separating sought-after therapeutic effects of cannabinoid receptor agonists from the unwanted consequences of CB1 receptor activation are emerging. Recently, ligands have been developed that are potent and selective agonists for CB1 and CB2 receptors, as well as potent CB1—selective antagonists and inhibitors of endocannabinoid uptake or metabolism.[60] In addition, varieties of cannabis are known to contain a mix of partial cannabinoid agonists and antagonists, which can be rationally used. This knowledge may lead to the design of synthetic cannabinoid agonists and antagonists as well as cannabis strains with high therapeutic potential. The fact that both CB1 and CB2 receptors have been found on immune cells suggests that cannabinoids play an important role in the regulation of the immune system. Recent studies show that cannabinoids downregulate cytokine and chemokine production, both mechanisms that suppress inflammatory responses.[61-64] Manipulation of endocannabinoids (ie, via the use of exogenous cannabis) has great potential treatment viability against inflammatory disorders, including the inflammation seen in the central nervous system (CNS) of the patients with ALS. The potential use of cannabinoids as a novel class of anti-inflammatory agents may become one of the predominant indications, as that includes not only neuromodulation but pain as well.[65,66] Indeed, any number of inflammatory processes that are at least partially triggered by activated T cells or other cellular immune components could be treated with cannabis and other cannabinoid-based medicines.

Cannabinoids are chemically classified as terpenes. These are lipid-soluble hydrocarbons that function as major biosynthetic cellular messengers in many forms of life. Terpenes are widespread in plants and most species of animals as well, including humans. Any compound that resembles the basic terpenes structure, yet may be modified chemically via oxidation or other processes, is termed a terpenoid. Many hormones, including estrogens, are terpenoids, and share the same basic organic chemical structure as cannabinoids.[53,54] All terpenes are organic, readily penetrate the highly lipophilic CNS.

Interestingly, tamoxifen, which is an antagonist of the estrogen receptor in breast tissue, is terpenoid and chemically resembles cannabinoids. Tamoxifen's primary use is as a FDA-approved drug for the treatment of breast cancer.[67-69] However, phase II clinical trials of tamoxifen in ALS have now demonstrated preliminary efficacy and safety.[68] A phase 2B study demonstrated increased survival after 2 years in patients with ALS taking higher doses of tamoxifen, with no effect seen in 2 lower dose groups.[68] The 3 higher dose groups experienced a 4- to 6-month prolongation of survival over a 24-month trial, with no significant side effects observed.[68] Interestingly, glutamate uptake in cultured retinal cells is inhibited by tamoxifen, thus this mechanism may be part of a possible beneficial effect in ALS.[67] The chemical similarity between cannabinoids and tamoxifen points to a possible shared mechanism of action for neural protection.[69]

The cannabis plant is a remarkably complex plant, with several phenotypes, each containing over 400 distinct chemical moieties.[70-73] Approximately 70 of these are chemically unique and classified as cannabinoids.[70-73] Delta-9 tetrahydrocannabinol (THC) and delta-8 THC appear to produce the majority of the psychoactive effects of cannabis.[74,75] Delta-9 THC, the active ingredient in dronabinol (Marinol), is the most abundant

cannabinoid in the plant, which historically led researchers to erroneously hypothesize that it was the main source of the drug's impact. It is now known that other major plant cannabinoids, including cannabidiol and cannabinol, modify the pharmacology of THC and have distinct effects of their own. Cannabidiol is the second most prevalent of cannabis's active ingredients and may produce most of its therapeutic effects. Cannabidiol becomes THC as the plant matures and this THC over time breaks down into cannabinol. Up to 40% of the cannabis resin in some strains is cannabidiol.[72] The amount varies according to plant. Some varieties of *Cannabis sativa* have been found to have no cannabidiol.[72] Cannabidiol breaks down to cannabinol as the plant matures. Much less is known about cannabinol, although it appears to have distinct pharmacological properties that are quite different from cannabidiol. Cannabinol has significant anticonvulsant, sedative, and other pharmacological activities likely to interact with the effects of THC.[75-78] Cannabinol may induce sleep and may provide some protection against seizures for epileptics.[78]

## Hypothetical Applications

### *Preclinical Studies of the Endocannabinoid System in ALS*

The primary murine model for human ALS is the G93A-SOD1 mutant mouse, which is genetically engineered to replicate familial ALS.[4] There is strong evidence in the G93A-SOD1 mouse model of ALS that the endocannabinoid system is involved, both directly and indirectly, in the pathophysiology of the disease. Several recent studies have highlighted this. Rossi et al[17] investigated both excitatory and inhibitory synaptic transmission in the striatum of symptomatic G93A-SOD1 ALS mice, along with the sensitivity of these synapses to CB1 receptor stimulation. They reported a reduced frequency of glutamate-mediated spontaneous excitatory postsynaptic currents and increased frequency of GABA-mediated spontaneous inhibitory postsynaptic currents in recordings from striatal neurons in ALS mice. This is likely due to some presynaptic defects in transmitter release. The sensitivity of CB1 receptors in controlling both glutamate and GABA transmission was potentiated in ALS mice. This provides good evidence that adaptations of the endocannabinoid system might be involved in the pathophysiology of ALS. This is not inconsistent with current theories on pathophysiological mechanisms of ALS, which still remain largely a pathophysiologic enigma.[79-83]

Bilsland et al[18] showed that treatment of postsymptomatic, 90-day-old SOD1G93A mice with a synthetic cannabinoid, WIN55,212-2, significantly delayed disease progression. Furthermore, genetic ablation of the fatty acid amide hydrolase (FAAH) enzyme, which results in raised levels of the endocannabinoid anandamide by preventing its breakdown, prevented the appearance of disease signs in 90-day-old SOD1G93A mice. Surprisingly, elevation of cannabinoid levels with either WIN55,212-2 or FAAH ablation had no effect on life span. Ablation of the CB1 receptor, in contrast, had no effect on disease onset in SOD1G93A mice but significantly extended life span. Together, these results indicate that cannabinoids have significant neuroprotective and disease-modifying effects in this model of ALS and suggest that these beneficial effects may be mediated by non-CB1 receptor-based mechanisms.

It is now known that during active neurodegeneration from disease or trauma in the CNS, the concentration of tumor necrosis factor alpha (TNF-α) rises well above normal levels during the inflammatory response. Addition of exogenous TNF-α, both in vitro and in vivo, to neurons has been shown to significantly potentiate glutamatergic excitotoxicity. Thus, the discovery of drug targets reducing excess TNF-α expression may help protect neurons after injury. Zhao et al[84] investigated the neuroprotective role of the CB1 receptor after TNF-α exposure in the presence or absence of CB1 agonists. They demonstrated that CB1 activation blocks the TNF-α-induced increase in inflammation, thus protecting the neurons from damage. Thus, neuroprotective strategies which increase CB1 activity may help to reduce damage to motor neurons in ALS that are mediated by CNS inflammation.

Additionally, CB2 receptors are dramatically upregulated in inflamed neural tissues associated with CNS disorders, including ALS.[85] In G93A-SOD1 mutant mice, endogenous cannabinoids are elevated in spinal cords of symptomatic mice.[21] Furthermore, treatment with nonselective cannabinoid partial agonists prior to, or on, symptom appearance minimally delays disease onset and prolongs survival through undefined mechanisms. Shoemaker et al[14] demonstrated that messenger RNA (mRNA) levels, receptor binding, and function of CB2, but not CB1, receptors are dramatically and selectively upregulated in spinal cords of G93A-SOD1 mice in a temporal pattern paralleling disease progression. Daily injections of the selective CB2 agonist AM-1241, initiated at symptom onset, increased the survival interval after disease onset by 56%.[14]

### *Disease-Modifying Treatment of ALS*

Clinical trials for ALS have been largely based on preclinical work using the G93A-SOD1 mouse. Unfortunately, translation of therapeutic success in mice to humans has proven quite difficult and a cure for ALS is not yet known. Many factors have been implicated in explaining the predominantly negative results of numerous randomized clinical trials in ALS, including methodological problems in the use of animal-drug screening, the lack of assessment of pharmacokinetic profile of the drugs, and methodological pitfalls of clinical trials in patients with ALS. Riluzole is currently the only agent approved by the FDA for the treatment of ALS.[10] This drug inhibits the presynaptic release of glutamate and reduces neuronal damage in experimental models of ALS. Four controlled trials of a total of 974 riluzole-treated and 503 placebo-treated patients showed that it prolonged survival opposed to placebo, although the benefit was fairly modest.[10] Because oxidative stress is one of the proposed pathogenic factors in ALS, antioxidants have been extensively tested, including vitamin E, vitamin C, coenzyme Q, B-carotene, *N*-acetylcysteine, and creatine, an amino acid naturally found in skeletal.[11] To date, trials of

neurotrophic factors, antioxidants, glutamate antagonists, and creatine have all failed to show any significant benefit in humans, although most had significant benefit shown in mice.[11] It is currently felt that a cocktail approach may be the ideal treatment strategy, including glutamate antagonists, antioxidants, and neurotrophic factors.[68] Recently, the kynurenine pathway (KP) has emerged as a potential target for ALS treatment.[8] The KP is a major route for the metabolism of tryptophan, generating neuroactive intermediates in the process. These catabolites include the excitotoxic *N*-methyl-D-aspartate (NMDA) receptor agonist, quinolinic acid (QUIN), and the neuroprotective NMDA receptor antagonist, kynurenic acid (KYNA). These catabolites appear to play a key role in the communication between the nervous and immune systems and also in modulating cell proliferation and tissue function. Targeting the KP, hence, could offer a new therapeutic option to improve ALS treatment, and several drugs that block the KP are already under investigation.

Although other potential neuroprotective agents have been evaluated in randomized clinical trials, none have shown unequivocal benefit for the treatment of ALS. Thus, there remains an enormous need for more trials to test other putative disease-modifying therapies. As the effectiveness of such drugs can only be definitively established by large, costly, phase III randomized controlled studies, it is imperative that researchers target compounds that have potential benefit based on demonstrated pharmacological and physiological mechanisms.

There remains the possibility that ALS could represent a state of clinical endocannabinoid deficiency (CED).[28,31] The endocannabinoid anandamide demonstrates dopamine-blocking and anti-inflammatory effects and is also tonically active in the periaqueductal gray matter.[81] Endocannabinoids also modulate glutamatergic neurotransmission indirectly via NMDA receptors, and these pathways can be modulated to produce a clinical effect, such as reduction in motor tone, seizure threshold, and perception of pain and mood state.[82-93] These clinical, biochemical, and pathophysiological patterns could reflect an underlying abnormality in the endocannabinoid system in ALS that could be potentially treated with exogenous cannabinoids, that is, via clinical use of cannabis or some derivative thereof.

## Practical Applications

### *Symptom Management in ALS*

As discussed previously, animal studies strongly suggest that the endocannabinoid system is implicated in the pathophysiology of ALS, either directly as part of the underlying disease mechanisms, or indirectly, inasmuch as this system plays a role in the homeostatic functioning of the neuromuscular system. Irrespective, it is clear that cannabinoids are able to slow down the progression of ALS in mice, likely by acting as an antioxidant, among other mechanisms.[15-18] In addition to the neuroprotective effect, patients report that cannabis helps in treating symptoms of the disease, including alleviating pain and muscle spasms, improving appetite, diminishing depression, and helping to manage sialorrhea (excessive drooling) by drying up saliva in the mouth.[24] Indeed, in a large survey it was noted that patients with ALS who were able to obtain cannabis found it preferable to prescription medication in managing their symptoms. However, this study also noted that the biggest reason patients with ALS were not using cannabis was their inability to obtain it, due to legal or financial reasons or lack of safe access.[24,26]

There are many other clinical problems faced by patients with ALS that could be helped by cannabis. The majority of patients with ALS experience significant pain.[24] The pain is largely due to immobility, which can cause adhesive capsulitis, mechanical back pain, pressure areas on the skin, and more rarely, neuropathic pain.[24,31] Pain in ALS is a frequent symptom especially in the later stages of disease and can have a pronounced influence on quality of life and suffering.[94-98] Treatment of pain, therefore, should be recognized as an important aspect of palliative care in ALS. A recent Cochrane review of the evidence for the efficacy of drug therapy in relieving pain in ALS revealed no randomized or quasi-randomized controlled trials showing significant benefit. Despite the major pain problems encountered by patients with ALS, there are no clear guidelines and few randomized clinical trials about how to manage pain in ALS. However, as noted previously, the cannabinoids have been shown to produce an anti-inflammatory effect by inhibiting the production and action of TNF and other acute phase cytokines.[35] Additionally, cannabis may reduce pain sensation, likely through a brain stem circuit that also contributes to the pain-suppressing effects of morphine.[99] Cannabinoids produce analgesia by modulating rostral ventromedial medulla neuronal activity in a manner similar to, but pharmacologically distinct from, that of morphine.[100,101] This analgesic effect is also exerted by some endogenous cannabinoids (anandamide) and synthetic cannabinoids (methanandamide) and may be prevented by the use of selective antagonists.[102-104] Thus, cannabinoids are centrally acting analgesics with a different mechanism of action than opioids, although the analgesia produced by cannabinoids and opioids may involve similar pathways at the brain stem level.[103-105]

There are now multiple, well-controlled clinical studies using cannabis to treat pain, showing ample evidence of analgesic efficacy.[106] A recent systematic review and meta-analysis of double-blind randomized controlled trials that compared any cannabis preparation to placebo among participants with chronic pain showed a total of 18 completed trials. The studies indicate that cannabis is moderately efficacious for the treatment of chronic pain.[106] In the setting of ALS, the medications should be titrated to the point of comfort. Concomitant use of narcotics may also be beneficial because, as noted above, the opioid receptor system is distinct from the cannabinoid system. In that regard, the antiemetic effect of cannabis may help with the nausea sometimes associated with narcotic medications.

In addition to pain, spasticity is also a major problem for patients with ALS. Spasticity in ALS is induced both at the

motor cortex and at the spinal cord level through the loss of motor neuron inhibition.[107-110] Cannabis has an inhibitory effect via augmentation of γ-amino-butyric acid (GABA) pathways in the CNS.[111] This produces motor neuron inhibition at spinal levels in mice. Several past studies have suggested that cannabinoid therapy provide at least a subjective reduction of spasticity, although virtually all of the studies have been done in patients with multiple sclerosis (MS).[29,112] A survey study has shown that patients with ALS do subjectively report that cannabis helps alleviate symptoms of spasticity.[24]

In addition to pain and spasticity, there are other pharmacological effects of cannabis that may be useful for patients with ALS. Patients with ALS and bulbar symptoms also usually have difficulty controlling and swallowing the saliva that is normally present in the oral cavity.[113] Cannabis is a potent antisalivatory compound that swiftly dries the oral cavity and upper airway, potentially reducing the risk of aspiration pneumonia and increasing patient comfort.[22,24]

Cannabis also increases appetite and may help prevent "ALS cachexia," a phenomenon experienced by some patients where weight loss occurs in excess of that caused by muscle atrophy and reduced caloric intake.[114-116] In addition to improving appetite, cannabis appears to also help with mood state and sleep. Patients with ALS previously have reported that cannabis is at least moderately effective at reducing symptoms of pain, spasticity, drooling, appetite loss, and depression.[24]

Cannabinoids will vaporize at temperatures in the range of 200°F and can be inhaled via a hot mist.[117-119] This delivers the cannabinoids rapidly, allowing for ease of titration and letting patients with ALS having severe dysarthria rapid access to the drug's effects. Vaporizing also helps dry up oral secretions.[24] Cannabis may also be ingested orally or through a feeding tube, although absorption is much slower. Cannabis can be titrated to desired effect, with individual, patient-specific dosing.[120-122] In terms of clinical trials for disease-modifying effects, dosing paradigm would be more complex. Fortunately, the low toxicity of cannabis would allow for trail and error. Based on the available studies, a typical dosing range for clinical effects would likely be 1 to 2 g/d of cannabis, with an average THC content of 20% by weight.[122]

## A Call for Clinical Trials

In terms of symptoms management, cannabis is a substance with many pharmacological properties that are directly applicable to the clinical care of patients with ALS. These include analgesia, muscle relaxation, bronchodilation, saliva reduction, appetite stimulation, sleep induction, and mood elevation.[24] From a pharmacological perspective, cannabis is remarkably safe with realistically no possibility of overdose or frank physical addiction. There is a valid, logical, scientifically grounded rationale to support the use of cannabis in the pharmacological management of ALS. Indeed, cannabis, as a single compound, could potentially replace and provide the benefits of multiple standard medications, including analgesics, antispasmodics, anxiolytics, antidepressants, appetite stimulants, and agents used to dry the mouth (typically anticholinergic medications). There is ample clinical evidence to warrant the empiric use of cannabis to manage the symptoms of ALS.

From an experimental, disease-modifying perspective, it is not likely that a single mechanism agent would treat all of the abnormal physiological processes occurring simultaneously in this devastating disease.[123-127] Thus, some experts are now advocating for a combination drug approach to slowing the progression of ALS.[80] Based on what is known about the pathophysiology of ALS, a multidrug regimen would include glutamate antagonists, antioxidants, a CNS anti-inflammatory agent, a microglial cell modulators, including TNF-α inhibitors, an antiapoptotic agent, 1 or more neurotrophic growth factors, and a mitochondrial function-enhancing agent.[127,128] Remarkably, cannabinoids appear to have at least some activity in all of those categories.[129-131] Moreover, there is a particularly strong, growing, body of preclinical data indicating that cannabis has powerful antioxidative, anti-inflammatory, and protective neuromodulatory effects.[132-135] In the G93ASOD1 ALS mouse, this has translated to prolonged neuronal cell survival.[15,16,18,43]

There is an overwhelming amount of preclinical and clinical evidence to warrant initiating a multicenter randomized, double-blind, placebo-controlled trial of cannabis as a disease-modifying compound in ALS. Secondary outcome measures could include clinical management, with end points such as pain scores, quality-of-life measures, and so on. Developing a multicenter clinical research trial using cannabis would pose many unique barriers that would have to be overcome. Inasmuch as there is no commercial manufacturer of cannabis, the study would have to be funded either by the federal government or privately. Presumably, there would be no industry funding. Obtaining the trial drug would require the investigators to gain access to a large, reliable supply of cannabis that is legal for medical research. At present, the only source of cannabis that can be legally used in research in the United States is through the National Institute on Drug Abuse (NIDA). National Institute on Drug Abuse provides low-potency material and makes the cannabis available only to projects it approves. National Institute on Drug Abuse supplies cannabis with a THC content, by weight, of 2% to 4% typically, although it has supplied cannabis with an 8% by weight THC content on occasion.[136,137] The average THC content of cannabis at randomly surveyed medical cooperatives in California is approximately 15% to 20%.[26,117,121] Thus, an independent source of cannabis would be needed to ensure a consistently high cannabinoid content that may be strong enough to possibly alter the disease progression. An independent cannabis source would also allow investigators to avoid NIDA's arbitrary and lengthy review process that it mandates before providing any cannabis for research. Historically, NIDA has derailed clinical trial plans by refusing to supply cannabis, even after the research protocols were approved by the FDA.[117] Nonetheless, it is possible, with coordinated effort, to effectively do double-blind, randomized, placebo-controlled clinical trials with cannabis.[138-141] To properly evaluate both subjective and objective

effects, cannabinoid blood levels should be followed as well, to further ensure adequate data for a dose–response curve.

Clinical trials with cannabis would also address the issue of single versus multiple drug clinical trials. Arguable, multiple drug trials would increase the chances of success but also exponentially increase the difficulty of completing the trial and analyzing the data. Cannabis, as a single agent, in essence provides the advantages of a multiple drug trial due to its multiple mechanisms of action. Cannabis is a unique compound that possesses significant internal therapeutic synergy. The search for the underlying cause of ALS continues.[142,143] With respect to treatment, from both a symptom management and disease modifying viewpoint, the logical next step, based on the available science, would be clinical trials with cannabis. Although not expected to be necessarily curative, it is not unreasonable to think that cannabis might significantly slow the progression of ALS, potentially extending life expectancy and substantially reducing the overall burden of the disease.

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Review Article

# A comprehensive review of amyotrophic lateral sclerosis

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## Abstract

Amyotrophic lateral sclerosis (ALS) is a late-onset fatal neurodegenerative disease affecting motor neurons with an incidence of about 1/100,000. Most ALS cases are sporadic, but 5–10% of the cases are familial ALS. Both sporadic and familial ALS (FALS) are associated with degeneration of cortical and spinal motor neurons. The etiology of ALS remains unknown. However, mutations of superoxide dismutase 1 have been known as the most common cause of FALS. In this study, we provide a comprehensive review of ALS. We cover all aspects of the disease including epidemiology, comorbidities, environmental risk factor, molecular mechanism, genetic factors, symptoms, diagnostic, treatment, and even the available supplement and management of ALS. This will provide the reader with an advantage of receiving a broad range of information about the disease.

**Key Words:** Amyotrophic lateral sclerosis, sporadic and familial ALS, superoxide dismutase

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## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disorder that is, characterized by progressive loss of the upper and lower motor neurons (LMNs) at the spinal or bulbar level.[251]

ALS was first described in 1869 by French neurologist Jean-Martin Charcot.[213,251,286,323] The disease became well known in the United States when baseball player Lou Gehrig was diagnosed with the disease in 1939.[165,213] ALS is also known as Charcot disease in honor of the first person to describe the disease, Jean-Martin Charcot, and motor neuron disease (MND) as it is one of the five MNDs that affect motor neurons. There are four other known MNDs: Primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy (PBP), and pseudobulbar palsy.[165,213,251,286]

ALS is categorized in two forms. The most common form is sporadic (90–95%) which has no obvious genetically inherited component. The remaining 5–10% of the cases are familial-type ALS (FALS) due to their associated genetic dominant inheritance factor.[2,107,289,299] The first onset of symptoms is usually between the ages of 50 and 65.[169,170,186,228] The most common symptoms that appear in both types of ALS are muscle weakness, twitching, and cramping, which eventually can lead to the impairment of muscles.[101,323] In the most advanced stages, ALS patients will develop symptoms of dyspnea and dysphagia.[164,238]



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Most of the reviews about ALS focus on a specific area of the diseases such as molecular mechanism, treatment, diagnostic, etc. This review will attempt to provide an up-to-date overview of all aspects of ALS. It will first cover the epidemiology and comorbidities of the disease, followed by known environmental risk factors such as smoking, chemical exposure, and radiation.

Improving the understanding of ALS pathogenesis is critical in developing earlier diagnostic methods as well as proposing new effective treatments. Thus, this review will present the most recent studies related to molecular mechanisms, genetics, ALS symptoms, diagnostic examinations, and treatments. Furthermore, due to the fact that there has been only one Food and Drug Administration (FDA) approved drug for ALS treatment, this review will also address nutritional supplements, as well as respiratory and nutritional managements that help alleviating the symptoms. This comprehensive study will inevitably lead to the better understanding of ALS and assist in extending the life expectancy associated with ALS by establishing a basis of knowledge that can be used to improve care.

## THE EPIDEMIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS

During 1990's, the number of reported cases of ALS was between 1.5 and 2.7 per 100,000 in Europe and North America.[170,228,326] Recent studies have shown that disease prevalence has not increased over the past decade, as the incidence rate remains at 2.7/100,000 (95% confidence interval [CI] 2.63–2.91)[146,169,228] and as of 2008, the prevalence of ALS was 0.32/100,000 (95% CI 9.78–10.86).[2,170,228,305] Multiple studies have shown an increased risk in the male to female ratio (male: female =1.5:1),[123,157,170,228,305] although other studies have shown a balance in this ratio.[2,168,326]

The mean age of onset of ALS varies from 50 to 65 years with the median age of onset of 64 years old. Only 5% of the cases have an onset <30 years of age.[169,170,228,286] ALS incidence is most pronounced in people 80 years or older (10.2/100,00 in men; 6.1/100,000 in women). One hypothesis for the increased incidence in older population is related to the variation in care for these patients.[2,93,146]

Although most cases of ALS are sporadic, about 5% of the cases have a family history. The age of onset for FALS is about a decade earlier than for sporadic cases.[2,107,289,299] There is also geographical loci form of ALS where prevalence is 50–100 times higher in certain locations than in any other part of the world. These population include parts of Japan, Guam, Kii Peninsula of Japan, and South West New Guinea.[157-159] Although this evidence is not concrete, it is believed that the increased incidence of ALS in these regions is due to environmental factors, specifically a neurotoxic nonprotein amino acid, β–methylamino-L-alanine (BMAA) in the seeds of the cycad *Cycas micronesica* produced by a symbiotic cyanobacteria in the roots of the cycad that are commonly found in these areas. It is hypothesized that patients in these regions who develop ALS have an inability in preventing BMAA accumulation.[34,139,206] More research is needed in South America to corroborate data for the rest of the continent, yet new studies show that the incidence of sporadic ALS in Uruguay is similar to those found within North America and Europe.[305,311] Thus, it is important to continue epidemiologic studies of ALS in areas where little work has been done to identify vulnerable populations within Africa, Central America, and South America.

A possible relationship between ALS and sports participation has been proposed but not demonstrated. In a cohort study of Italian professional football players, a severe increase in the incidence of ALS was found.[1,5,48] This study, conducted by the National Institute of Environmental Health Science, showed a correlation between head injuries in football players and an increased risk of ALS (odds ratio [OR] =3.2; 95% CI =1.2–8.1). Traumas to other parts of the body were not associated with increased risk.[46,48]

## ANALYSIS OF CO-MORBIDITIES OF AMYOTROPHIC LATERAL SCLEROSIS

Retrospective cohort studies have shown that the incidence in certain concomitant diseases and comorbidities is significantly different in ALS-affected population in comparison to the general population. German cohort studies examining comorbidities prior to diagnosis found that while cardiovascular risk factors were the most common comorbidities in ALS patients (31.5% vs. 40%), they still had a significantly higher incidence in the general population than in their affected cohort.[105,127,151] The discrepancy in incidence could suggest that ALS causes a protective mechanism for cardiac disorders or that there are genetic benefits for those predisposed to the disorder. ALS patients had a significantly lower incidence of arterial hypertension (31.5% vs. 47.2%), coronary heart disease (8.6% vs. 9.3%), myocardial infarctions (6.4% vs. 7.0%), diabetes mellitus (7.2% vs. 10.6%), and hypercholesterolemia (17.9% vs. 65.6%).[105,151,325] These results were echoed when results were stratified by various age groups to prevent confounding. With regards to comparing ALS types and their onset time to patient comorbidities, only a difference in hypercholesterolemia incidence was seen when comparing bulbar onset to spinal onset (23.6% vs. 15.7%; $P < 0.05$).[151] This shows only a slight association with how cardiovascular comorbidities can alter ALS progression.

Of the diseases most likely to be found in ALS patients prior to their diagnosis, a higher incidence in neurological disorders was noted when compared to the general population.[127,151] The increased incidence of neurological diseases in ALS patients may be indicative of similar underlying genetic factors between ALS and other neurological disorders. A history of depression (22.8% vs. 11.6%), dementia (5.8% vs. 1.3%), Parkinson's (1.8% versus 0.1–0.2%), and epilepsy (1.6% versus 0.45–1%) was found more frequently within the study cohort than with the rest of the population.[38,77,151] The results were echoed in other studies that have indicated a greater likelihood of ALS development and progression when examining patients with a high rate of psychological disorders.[189]

Development of depression is one of the most common secondary symptoms associated with ALS. Previous studies have reported a prevalence of depression of 4–56% depending on the assessment measure.[24,95,113,242,287,322] Depression has a negative effect on the quality of life of patients with ALS.[52,156,173] In a recent study, 131 patients with ALS were evaluated to estimate the prevalence of depression.[116] The results showed 29% prevalence of mild depression and 6% prevalence of severe depression.[153] In this study, more than one-third of ALS patients were receiving antidepressant to treat sialorrhea, pseudobulbar affect, and insomnia, which may explain the lower rates of severe depression in ALS patients compared to the prevalence of depression in the general population (10%).[12,153] Physical impairment and duration of the disease did not predict depression in the cohort which suggests that depression is not related to advanced ALS or approaching end of life.[153] ALS-related symptoms, including cramps, stiffness, shortness of breath, swallowing difficulty, insomnia, loss of appetite, increased saliva, uncontrolled laughing or crying, fasciculations, anxiety, fatigue, and pain, did not appear to contribute to depression with the exception of anxiety which is a known symptom of depression.[153] Other studies have also shown that, even in late stages of ALS, major depression is rare.[102,246]

When comparing the survival rates of patients with mental health comorbidities, it was noted that patients suffering from Parkinson's and ALS had a slower progression of their disease and a greater survival time ($P = 0.03$).[151] It may be possible that the pathological development of Parkinson's disease (PD) could slow down the effects of ALS onset by altering the speed of nervous degeneration and disease progression. It is also possible that the administration of L-DOPA or the decreased levels of endogenous dopamine could offer an antioxidant protective effect in those susceptible to ALS, similar to that seen in multiple studies comparing ALS incidence and Vitamin E consumption.[279,315] Greater evidence will be necessary to confirm correlations and association between these comorbidities and ALS incidence.

## AMYOTROPHIC LATERAL SCLEROSIS ENVIRONMENTAL FACTORS

Previous epidemiologic studies have suggested that ALS patients may have been exposed to environmental toxins.[329] Exposures to agriculture chemicals, heavy metals, solvents, electrical magnetic fields, type of diet, dust/fibers/fumes, and physical activity were all examined for association with ALS.[211,329] The following section will discuss more in depth the role that each of these risk factors have with increased ALS incidence.

## SMOKING

Cigarette smoke has been found to increase the probability of developing ALS through inflammation, oxidative stress, and neurotoxicity by heavy metals contained in cigarettes.[317,318] Among those that actively smoke, ALS risk is highest when they start smoking at a younger age.[318] It is not, however, associated with duration or intensity of smoking habits. Furthermore, exhaled cigarette smoke contains formaldehyde which is associated with higher mortality rates in ALS patients.[319] It is thought that cigarette smoking is the most consistent nongenetic risk factor for ALS.[318,319] Finally, a beneficial effect of quitting smoking on ALS patient has not been examined so far.

### Physical activity

Athletes have higher ALS risk compared to the general population; however, performing passive to robust physical activity has not shown an increased susceptibility of developing ALS.[123] Nevertheless, there are several genes (i.e., ciliary neurotrophic factor, leukemia inhibitory factor, and vascular endothelial growth factor 2) related to exercise that have been recognized as possible ALS risks factors.[47] Moreover, some studies have obtained inconsistent results in regards to high ALS risk among athletes, thus invalidating any association between physical activity and ALS risk.[17] Hence, the physical activity itself is not yet proven to be a cause of ALS. A possible explanation to the high risk of ALS incidence among athletes involves genetic profiles.

Genetic profiles that promote physical fitness but not necessarily muscles strength could hold a proportional correlation between ALS and physical activity.[298] In other words, a genetic profile altered by exogenous factors promoting physical fitness increases ALS susceptibility.[52,298] This idea is supported by findings of a beneficial vascular risk profile in patients and their relatives.[123] They reported a reduced frequency

of coronary heart disease premorbid to ALS and an increased risk of ALS alongside physical fitness but not muscle strength, pointing at a mutual element between physical/cardiovascular fitness, and ALS susceptibility.[123,185,298,322]

### Chemical exposure and metals

ALS has shown an association with exposure to agricultural chemicals such as pesticides, fertilizers, herbicides, insecticides, and formaldehyde.[319,329] In a prospective study, it was found that people who reported 4 or more years of exposure to pesticides/herbicides might be at an increased risk of acquiring ALS, but no association was found between mortality rate and amount of exposure. This study also found that among individuals with long period of exposure to formaldehyde, the ALS death rate was more than 2 times higher compared to those unexposed one.[319] Also, as formerly mentioned formaldehyde is a byproduct of cigarette smoke, this may account for 10–25% of indoor air formaldehyde exposure.

Among all the heavy metals that might be associated with ALS, lead exposure seems to be studied the most possibly due to the ALS-like symptoms experienced by people exposed to high concentrations of lead.[138] Since then, recent studies have found a correlation between lead exposure and ALS.[191] As such, professions related to lead exposures, such as welding, have demonstrated a significant association with developing ALS with odds ratio (ORs) ranging from 1.9 to 5.7.[133,191,261]

It is thought that lead's role in ALS has to do with its ability to substitute for calcium in intracellular reactions leading to damage the mitochondria, oxidative damage to neurons, and strengthen glutamate's excitotoxicity.[261,271] However, most case–control studies established the lead and ALS association through self-reported occupational lead exposure. When a group of researchers utilized an expert evaluation panel of industrial hygienists to examine self-reported occupational lead exposures, no association was found between ALS and lead exposure.[191] This suggests that recall bias might have interfered with collected data.[133,138] Hence, more studies are needed in order to establish fully this relationship.

### Radiation/electromagnetic fields

Laboratory studies have demonstrated that *in vitro* exposures to extremely lowfrequency electromagnetic waves generate a bigger quantity of cellular reactive oxygen than normal.[269] *In vivo*, the same exposure produces oxidative stress and disables the antioxidant properties cells might have.[182] This oxidative damage can lead to ALS since oxidative stress has a role in ALS pathogenesis.[14] In fact, studies have observed that electromagnetic fields cause DNA strands to break in brain cells, leading to cell death (apoptosis and necrosis).[236] Such reaction could be the reason for the association between electromagnetic fields and ALS risk.[236] However, none of the current studies found a conclusive connection among electromagnetic field exposure, oxidative stress in neurons, and/or ALS development.[330]

*Diet*

Previous studies state that consuming high level of glutamate and fat can have adverse effects on ALS patients while Omega 3 fatty acids, Vitamin E, and fiber can have defensive impact.[211,306] According to previous studies, overstimulation of glutamate receptors leads to high intracellular calcium levels, which can initiate selective neuron death similar to ALS mechanism.[130] Glutamate is found in protein-rich foods, tomatoes, mushrooms, milk, and cheese.[216] Normally glutamate does not cross the blood-brain barrier, hence it is not known if dietary glutamate affects neurotransmission.[276] Moreover, there are areas of the brain called circumventricular organs, which are susceptible to plasma glutamate levels.[276] Omega 3 has been known to possess anti-inflammatory characteristics, which in turn would theoretically reduce inflammation caused by neuronal death.[76] Omega 3 in conjunction with Vitamin E has been reported to reduce ALS risks up to 60%.[306] These nutrients appear to act together in a summative way.

A large number of the information regarding environmental factors are based off questionnaires, all of which rely on subjects' memories, leading to recall bias. Because of this, there may be a lack of information about the frequency and the amount of exposure to environmental factors. Also, this may also lead to the absence of biological markers in order to validate patient claims of exposure or pinpoint the possible action site.

Furthermore, due to ALS prolonged onset, it is difficult to isolate an exact environmental factor. In order to identify or narrow down possible ALS risk factors, a cohort study utilizing mice as a control and experimental group could be appropriate. Starting out with an emphasis on the most sought out factors (smoking, heavy metals, physical activity, diet, radiation, and chemical exposure). In order to track changes accurately, a type of biomarker specific to the possible risk factor could be designed, to theoretically track the progression of the disease.

## MOLECULAR MECHANISM

Finding the molecular mechanisms by which motor neurons degenerate in ALS will aid in better understanding the disease's progress. Also, elucidation of molecular mechanisms can yield insight into developing strategies for newer treatments. The molecular basis of ALS is an intriguing issue that warrants in-depth research and investigation.

The most common cause of ALS is a mutation of the gene encoding the antioxidant enzyme superoxide dismutase 1 (SOD1).[63,70,129,233,304] Mutant SOD1 has a structural instability that causes a misfold in the mutated enzyme, which can lead to aggregation in the motor neurons within the central nervous system (CNS).[94] Several hypotheses have been proposed in regards to the mechanism underlying the mode of action of mutant SOD and the subsequent neurodegeneration seen in ALS. The most important proposed hypothesis for the pathogenesis of ALS includes glutamate excitotoxicity structural and functional abnormalities of mitochondria, impaired axonal structure or transport defects, and free radical-mediated oxidative stress.[69,78,94,132,178,207,265,317,331] Even though these mechanisms play a critical role in neurodegeneration, they all are considered as secondary events in the causes behind ALS onset.[312]

## Glutamate excitotoxicity

Glutamate is synthesized in the presynaptic terminal. Uptake of glutamate into synaptic vesicles is facilitated by vesicular glutamate transporters.[266] During a normal neurotransmission process, glutamate is released into the synaptic cleft, where it activates postsynaptic receptors. Upon release of the vesicle, glutamate is removed from the synaptic cleft by several glial and neuronal cell transporter proteins, such as excitatory amino acid transporters (EAATs).[283] This continuous release and removal of glutamate maintain a concentration gradient balance and avoid the induction of excitotoxic neuronal damage.[263]

The motor cortex and spinal cord of ALS patients and transgenic SOD1 mouse model were found to have reduced astroglial glutamate transporter EAAT2, which leads to increased extracellular glutamate, over-stimulation of glutamate receptors, and excitotoxic neuronal degeneration.[167,297] Furthermore, this causes an excessive influx of calcium, excessive firing of motor neurons, and initiation of several destructive biochemical processes within the cell, which are all known as important pathophysiological processes in familial and sporadic forms of ALS.[126,313] Glutamate excitotoxicity contributes to the neurodegeneration either through activation of $Ca^{2+}$-dependent enzymatic pathways by increasing the influx of $Na^{+}$ and $Ca^{2+}$ ions or by the generation of free radicals.[88,94,125]

Aberrant EAAT2 messenger RNAs (mRNAs) were found in neuro-pathologically affected areas and cerebrospinal fluid (CSF) of ALS patients. These abnormalities included intron-retention and exon-skipping. According to these findings, aberrant mRNA is the main reason for the decrease in EAAT2 receptors among ALS patient.[120,167]

## Structural and functional abnormalities of mitochondria

In addition to glutamate excitotoxicity, mitochondrial dysfunction also plays an important role in the motor neuron degeneration. Mitochondria are membrane bound organelles that have a significant role in vital processes such as intracellular energy production, cellular respiration, calcium homeostasis, and control of apoptosis.[94] Accumulating evidence suggests that abnormalities in mitochondrial morphology and biochemistry contribute to the pathogenesis of ALS. Functional defects and altered mitochondrial morphology such as fragmented network, swelling, and augmented cristae were found in soma and proximal axons of skeletal muscle and spinal motor neurons of ALS patients.[25,53,83,178,180,259,307]

In the spinal cords of ALS patients, mutant SOD1 is deposited on the cytoplasmic face of the outer membrane and matrix of mitochondria.[233,304] The increase of misfolded mutant SOD1 in spinal cord mitochondria is considered as the main reason for mitochondrial dysfunction that leads to abnormal functioning of ATP production, calcium homeostasis, axonal transport of mitochondria, and apoptotic triggering.[25,168]

Mitochondria act as the powerhouse of every cell by converting energy into ATP that is, essential for the metabolism of the cells. Disturbed energy homeostasis and ATP deficits have been reported in the skeletal muscle biopsies of ALS patients. The normal process of electron transport chains is perturbed by the presence of mutant SOD1, causing less production of ATP. Some studies have demonstrated a decreased activity of respiratory chain complexes I and IV that are associated with defective energy metabolism.[234]

In addition to energy homeostasis, another major function of mitochondria in neurons regards buffering cytosolic calcium levels. Thus, unraveling the relationship between aberrant mitochondria, calcium dysregulation, and neuronal death is critical for the understanding of ALS pathogenesis. Calcium is one of the most significant intracellular messengers that play an important role in the regulation of metabolic pathways, neuronal development, and synaptic transmission. Mutant SOD1 has been found to disrupt calcium homeostasis. Several studies have shown that intracellular calcium is misregulated in ALS patients. A lower cytosolic $Ca^{2+}$ buffering ability has been found as a principal risk factor for motor neuron damage.[7,16,131]

Several studies reported the loss of $Ca^{2+}$ binding proteins such as calbindin-D28K and parvalbumin in the motor neurons of ALS patients.[6,21,185] These findings are in agreement that with studies showing neurons lost early in the ALS development have low cytosolic $Ca^{2+}$ buffering capabilities due to the loss of $Ca^{2+}$ binding proteins. Meanwhile increasing the cytosolic $Ca^{2+}$ buffering capacity has shown to reduce motor neuron degeneration.[16,132,196,302]

Regulating mitochondrial transport along axons is an essential task for the survival of neurons due to the

mitochondria's key role in ATP generation, calcium buffering, and apoptotic signaling. Mitochondria are constantly being transported and docked at the same time in areas with high demand of ATP and calcium homeostasis such as growth cones, nodes of Ranvier, and synaptic terminals.[178,331] Thus, any defects in mitochondrial transport will lead to energy depletion and disruption in $Ca^{2+}$ buffering, activating synaptic dysfunction and a loss of neurons. Several laboratories have identified disrupted axonal transport of mitochondria in ALS patients.[31,210] The axonal transport alteration impairs the degradation and recycling process of abnormal mitochondria, thus increasing the amount of dysfunctional mitochondria at distal axons.[178,229,233] Moreover, mitochondrial movement can also be suppressed in both anterograde and retrograde directions.[70,178,265]

Increased mitochondrial transport may slow axonal degeneration by delivering healthy mitochondria to axons while removing the damaged one from distal synapses.[264,331] Mitochondria have been an attractive target for ALS therapy development and drugs, such as olexisome, are already in clinical trial for ALS patients.[88]

Finally, mutant SOD1 aggregates may also interfere with components of mitochondrial-dependent apoptotic machinery, such as B-cell lymphoma 2 (Bcl-2), which is a regulator protein that controls cell death.[184,280] Thus, causing the stimulation of premature apoptotic cascade activation is leading to the release of cytochrome C in the presence of Bcl-2, which directly contributes to neuromuscular degeneration and neuronal dysfunction.[25]

### Impaired axonal structure and transport defects

Motor neurons are highly polarized cells with long axons that can be more than a meter in length and are thus vulnerable to damage. In addition to transmitting nerve impulses axons also transport organelles, RNA, proteins, lipids, and other cell parts to the axonal compartments. Moving toward the soma is called retrograde and is performed by cytoplasmic dynein molecular motors while moving toward the synaptic structures at the neuromuscular junction is an anterograde transport and is conducted by microtubule-dependent kinesin.[70]

Axonal transport in ALS patients is compromised. Dysregulation of axonal transport and the axonal compartment play a critical role in the pathophysiology of ALS. In several experiment with mutant SOD1 mice, loss of neurotrophic signaling and defective axonal transport were observed early in the disease process.[88,145,324] Both anterograde and retrograde transport were impaired by the presence of mutant SOD1.[22,70]

Several pathways may be responsible for the impaired axonal transport in cases with mutant SOD1. Some of the most important mechanisms involve defective mitochondrial function or energy depletion, disruption of kinesin function by tumor necrosis factor, and excitotoxic damage by glutamate.[69,142,291] Defective axonal transport causes an accumulation of neurofilaments, mitochondria, and autophagosomes in degenerated motor neurons. This leads to further hindrance of axonal transport and eventual motor neuron death.[124]

### Free radical-mediated oxidative stress

Reactive oxygen species (ROS) or free radicals form as natural byproducts of the normal metabolism of oxygen.[75] ROS accumulation causes severe damages to cell structures. The term oxidative stress is used to define a disturbance in the balance between the production ROS and cell's antioxidant defenses.[94] Losing the ability to detoxify the harmful reactive intermediates will lead to cell demise.

Increased oxidative damage has been reported in ALS case biopsies and altered redox reactions were among the earliest theories of how mutant SOD1 could cause cytotoxicity.[41]

SOD1 is a major antioxidant protein, thus a mutation in this gene could cause cytotoxicity. Elevation of free radicals and increased oxidative damage were found in CSF, serum, and urine samples of ALS patients.[267,272,277] In addition, oxidative damage to RNA species was found in both mutant SOD1 mouse models, as well as in human CNS biopsies.[44]

## GENETICS OF AMYOTROPHIC LATERAL SCLEROSIS

Sporadic ALS accounts for the majority of the cases of ALS, but genetic causes have been known to play a role.[39] FALS occurs due to mutations in specific genetic loci.[73] The inheritance follows a clear Mendelian pattern and is primarily autosomal dominant.[118,146]

The clinical and pathological presentation of FALS and sporadic ALS are similar. Genetic testing can be used to differentiate inherited versus sporadic ALS and also to rule out other diseases that clinically mimic ALS.[35,115,171] Hence, genetic testing enables researchers to categorize and conceptualize the disease and will aid in mapping the various genetic mechanisms of each type of ALS.[251]

Starting with the discovery of mutations in the SOD1 gene, which codes for copper/zinc ion-binding SOD, 18 other genes have been identified in association with FALS.[73,239,308] The additional genes that are known to cause FALS include: TARDBP, encodes TAR DNA-binding protein 43 (TDP-43); FUS, which codes for fusion in sarcoma; ANG, which codes for angiogenin, ribonuclease, and the RNAase A family 5; OPTN, which codes for optineurin; and C9orf72.[23,49,56,100,135,160,179]

SOD1 mutations, which account for 20% of cases of FALS and 5% of SALS, cause cytotoxicity, which still has an unclear pathophysiology.[146,254,260] TARDBP mutations represent 5–10% of FALS mutations.[146] TDP-43 and FUS, which represents 5% of FALS mutations, are part of the process of gene expression and regulation including transcription, RNA splicing, transport, and translation, as well as processing small regulatory RNAs.[146] ANG, responsible for the remaining 1% of FALS, is a gene, coding for an angiogenic factor that responds to hypoxia. OPTN is a gene involved in open-angle glaucoma, where a mutation in this gene eradicates the inhibition of nuclear factor kappa-beta activation, changing the distribution of OPTN in the cytoplasm.[146] Approximately, 50–60% of FALS patients have mutations arising from the 19 genes that have been identified to date.[4] SOD1 and C9orf72 mutations most often cause FALS, but their rates vary across population.[4]

FALS is inherited at a rate of 5–10% for all cases of ALS where family history of the disease is known.[73,146] In the United States, a founder effect has been identified for the A4V mutations in SOD1, whereas in Europe, this mutation is uncommon.[39,255] OPTN mutations occur most often in Japanese population.[39] To date, there has been no evidence for geographic variability in FUS and TARDBP.[39] As soon as more causative genes are identified in FALS, mutation frequencies across different FALS population will be available. The lifetime risk of ALS is 1:450 for women and 1:350 for men.[39] As family size increases, there is a greater likelihood of two family members having SALS.[39]

Close to 50% of FALS cases can be attributed to specific genes, and most are seemingly rare, highly penetrant, *de novo* mutations within affected families. Genome-wide association studies (GWAS) has allowed for the identification of common variables that are coupled to this disease.[118] Another technique is next-generation sequencing (NGS), otherwise known as massively parallel sequencing, which provides a way to map mutations for single gene diseases.[118,245] Together, GWAS and NGS have helped to identify genetic variables that are seen in parallel with a higher risk for developing ALS.[118] Ascertaining accurate clinical phenotypes is essential for the success of these techniques to avoid false positive results.[96,118]

Family aggregation studies for SALS patients have shown that many people who have common neurodegenerative disorders also have ALS, possibly indicating the presence of a susceptible gene that could be responsible for increasing neurodegeneration in kindreds.[146]

Many GWAS for SALS have resulted in identifying genes that are associated to the ALS disease.[146] Two new susceptible loci, 19p13.3 (UNC13A) and 9p21.2, were identified through collaborative research that combined study pools to elucidate effectively both genes.[146,303] If more research groups work on collaborative efforts, it is highly probably that more molecular pathways and genetic markers could be identified.[146]

## AMYOTROPHIC LATERAL SCLEROSIS SYMPTOMS

The different ALS phenotypical expressions are classified mainly as: Limb-onset ALS with a combination of upper motor neuron (UMN) and LMN signs in the limbs; bulbar onset ALS, characterized with speech and swallowing difficulties followed by limb weakening in later stages of the disease; PLS with pure UMN involvement; and finally, PMA with pure LMN involvement.[146,312,323] The main clinical feature in ALS is a combination of UMN and LMN damage involving brainstem and multiple spinal cord innervation regions. Limb-onset ALS is the predominant type with 70% of the cases among patients. Bulbar onset accounts for 25% of the cases, with the final 5% of the cases having initial trunk or respiratory involvement.[146]

ALS patients experience localized muscle weakness that begins distally or proximally in their upper and lower limbs. Usually, the onset symptoms are asymmetric and develop in progressive generalized weakness and wasting of the muscles. The majority of the patients develop bulbar and respiratory symptoms and spasticity, which affects manual dexterity and gait.[101] Pseudobulbar symptoms including emotional lability and excessive yawning have been observed in a substantial number of cases.[323] About 5% of the patients with respiratory weakness usually do not show significant limb or bulbar symptoms.[66] Instead, these patients present type 2 respiratory failure or nocturnal hypoventilation including dyspnea, orthopnea, disturbed sleep, excessive somnolence in daytime, morning headaches, anorexia, decreased concentration, and irritability or mood changes.[238] Muscle atrophy, including muscles of the hands, forearms or shoulders, and proximal thigh or distal foot muscle in lower limbs, is usually discovered early in the development of limb-onset ALS.[312]

Speech disturbances tend to appear before the development of dysphagia for solids and liquids. Symptoms characteristic of limb-onset can develop simultaneously with bulbar symptoms occurring within 1–2 years. Patients with bulbar symptoms suffer from sialorrhea (excessive drooling) due to difficulty of swallowing saliva and minor bilateral lower facial weakness from UMN damage. The generalized weakness of the lower half of the face causes difficulty with lip seal and blowing cheeks.[323] The rest of the cranial nerves remain intact; however, in very rare cases of late stage ALS disease, patients may develop supranuclear gaze palsy that is a neurodegenerative disorder that causes severe

balance problem and gaze dysfunction accompanied cognitive dysfunction.[222]

With the progression of ALS, patients develop the distinctive feature of a combination of upper motor and LMN degeneration signs within the same CNS region.[103] This affects the bulbar, cervical, thoracic, and lumbar areas. The main cause of death in ALS is respiratory failure as the result of pulmonary complications.[54] Patients, who undergo tracheostomy-delivered assisted ventilation to prolong their lives, eventually develop a state motor paralysis known as a "totally locked-in state" (TLS), which involves paralysis of all voluntary muscles and varying degrees of oculomotor impairment.[117]

Some uncommon symptoms of ALS include cramps and fasciculations in the absence of muscle weakness, and frontal lobe-type cognitive dysfunction.[323] Other atypical ALS types might also include weight loss, which is an indicative of a poor prognosis.[87] Patients notice the appearance of fasciculations and cramps before weakness and wasting of muscles, which have subtle onset and are exacerbated by cold temperatures. Fasciculations can be observed in various muscle groups while spasticity is observed in the upper limbs with increased tone and a supinator "catch." In the lower limbs, a patellar "catch" and clonus is seen along with hypertonia.[323]

Weakness, spasticity, and abrupt deep tendon reflexes are usually characteristic of UMN disturbances involving the limbs. LMN features, on the other hand, include fasciculation, wasting of the muscle, and weakness. Spastic dysarthria characterized by slow, labored, and distorted speech is a consequence of bulbar UMN damage.[80] In bulbar onset ALS, the gag reflex is preserved and abrupt. In contrast, the soft palate reflex may be weak. Symptoms that identify bulbar LMN damage include tongue weakening, wasting, and fasciculations along with flaccid dysarthria.[150] Flaccid dysarthria and palatal weakness ultimately produce nasal speech.[80]

In the majority of the cases, tendon reflexes become pathologically abrupt in a symmetrical pattern.[85] Examples include finger jerks in the upper limbs and a positive crossed adductor reflex in the lower limbs. Tendon reflexes might spread beyond the stimulated muscle group in an abnormal way. Hoffmann's sign shows a plantar stimulation of the extensor muscles and a positive sign in upper limbs.[252] In patients presenting a bulbar defect, dysarthria may develop as a consequence of LMN pathology or pseudobulbar palsy from UMN disorder, which leads to dysarthria of speech.[81] In initial stages of the disease, this may only be apparent after ingestion of small amounts of alcohol.[205]

In late stages of ALS, some patients develop flexor spasms or involuntary spams due to excess of activation of the flexor arc in spastic limbs.[219] Patients have reported bladder dysfunction with the urgency of micturition, sensory symptoms, and cognitive symptoms along with multisystem involvement.[26]

Other common symptoms in ALS are fatigue and reduced exercise capacity. As the disease progresses, patients require assistance with basic daily activities.[114] Dysphagia develops with consequent weight loss and malnutrition.[244] In late stages of the disease, patients may develop respiratory complications such as dyspnea, orthopnea, or hypoventilation, which results in hypercapnia and early morning headaches.[164] Progressive weakening of the respiratory muscles develops into respiratory failure, which is often triggered by pneumonia.

The symptoms of ALS can be further divided into primary and secondary symptoms. Primary symptoms include muscle weakness and atrophy, spasticity, speech disturbances, poor management of oral secretions, difficulty swallowing, and respiratory complications that result in death. Secondary symptoms usually accompany primary symptoms, and they can significantly reduce the quality of life of patients, such as pain or difficulty performing daily tasks.[114]

Even though pain has not been associated with ALS, it has been reported in nearly 70% of ALS patients at some point during the course of the disease.[217,223] Pain is classified as acute or chronic depending on duration and presence of abnormalities affecting how nerves transmit electrical impulses to the CNS.[79,198] Pain in ALS is mostly related to musculoskeletal conditions including muscle cramping and spasticity. Acute pain and chronic pain have been linked to ALS. It has been reported that musculoskeletal pain in ALS develops secondary to muscle atrophy and reduced muscle tone. This can arise as a consequence of damage to bones, tendons, ligaments, joints, nerves, and the affected muscle.[114] Muscle wasting in ALS incites collateral axonal sprouting that enhances the surviving units, creating an enlarged plate zone, and a less synchronized motor unit action potential.[258] A progressive dissociation of the mechanical and electrical properties of muscle is observed over time. This alteration in muscle coordination and force generation causes abnormal stress on the ligaments, tendons, and joints.[72,109,230] Continual muscle wasting and injury produce a decrease in strength, coordination, and tone leading to pain development. Contrary to pain onset, which usually occurs in late stages of ALS, cramps and fasciculations are more frequent at initial stages. Even though patients experience fasciculations before the onset of muscle weakness, concern arises after diagnosis.[275]

Spasticity in ALS is usually due to changes in UMN within the motor cortex. Alteration in UMN processing can create the primitive reflex, also known as the Babinski sign, an important sign of neuropathy.[275] Spasticity may

not necessarily produce pain, but it can induce painful cramps, alter manual dexterity and cause muscle fatigue. Other consequences of spasticity include involuntary mobilization of stiff joint, muscle contractures, pressure pain, and decubitus ulcers due to immobility and skin breakdown in flexor creases.[26-28,257] All of these changes can alter posture, range of motion, ambulation and gait, thus creating new sources of pain.[217]

## DIAGNOSING AMYOTROPHIC LATERAL SCLEROSIS

The complexity and heterogeneous nature of ALS makes early and accurate diagnose a continuous challenge.[8] There is an average delay of 13–18 months from the onset of a patient's symptoms to confirmation of the diagnosis.[51,84] The lack of an established biological marker for ALS, the highly variable initial clinical presentations of the disease, and its pathogenic overlap with several neurodegenerative disorders all contributes to the difficulty in diagnosing ALS with acceptable certainty.[65] ALS is primarily a clinically diagnosed disease based on the exclusion of other causes of progressive UMN and LMN dysfunction.[65,115] There are standard criteria and diagnostic tests that help rule out many of the differential diagnosis of ALS. This process includes obtaining a thorough patient history, conducting a thorough examination, appropriate laboratory, electrodiagnostic, and neuroimaging studies, as well as genetic testing.[35,65,115,121,208]

### Criteria and requirements for diagnosis

The El Escorial criteria for diagnosing ALS was published in 1994 by the World Federation of Neurology for inclusion standards for patients entering research studies and clinical trials.[3,35] The importance of laboratory exams as diagnostic tools to exclude differential diagnosis was included in a revised criteria and renamed to the Airlie House Criteria in 1998.[35,262,295] These two criteria are used to predict the degree of certainty of diagnosis and are also used as inclusion criteria for clinical trials and research purposes.[115,171,262] The Awaji algorithm was incorporated in 2000 and includes neurophysiological measurements of LMN degeneration while UMN dysfunction remains clinically based.[57,262] The Awaji criteria place equal emphasis on both electromyogram (EMG) and clinical abnormalities.[203] Several follow-up studies have shown that using the Awaji algorithm has successfully increased the ability to detect patients with ALS without increasing the number of false-positives.[57,65,97,195,262] As a result, patients can benefit from treatment and the corresponding results of the clinical trials. These criteria are based on the probability of the disease and do not take into consideration the behavioral and mental variations of ALS patients.[35,115]

A definitive diagnosis of ALS requires evidence of LMN and UMN degeneration, and progression and spread of neurological symptoms or signs within or toward another anatomical region.[115] The electrophysiological, laboratory, and neuroimaging results should not show evidence of other pathological processes that could explain the observed clinical presentation and exclude ALS as a cause.[35]

### Variability in clinical presentation

Based on the onset of symptoms, ALS is categorized as either a bulbar or spinal-onset disease, and further phenotypic subclassification is based on the extent of UMN and LMN dysfunction.[115,154] PLS, PMA, and PBP mimic the phenotype of ALS but vary in severity of the disease and prognosis.[115] PLS is defined as an UMN disorder and diagnosed in patients who have only UMN involvement and are classified as sporadic adult onset if the symptoms have been ongoing for more than 4 years.[115,249,274] Spinal signs are typically the first to manifest in PLS and develop into ALS in 77% of patients within 3–4 years.[115] It is especially important to differentiate PLS from ALS because the median survival of patients with PLS is >20 years, for those who do not develop ALS, whereas the average survival after onset of symptoms of ALS is approximately 3–5 years.[115,177,295] PMA involves LMN signs only, and 30% of the patients with PMA develop UMN signs within 18 months and continue to develop ALS.[115,310] PBP initially presents with affected speech and swallowing because of the LMN involvement of cranial nerves IX, X, and XII.[115] LMN syndromes with the segmental distribution of muscle involvement and disease duration of >4 years have an encouraging prognosis.[115,301] Patients with segmental disease phenotypes that were followed in a prospective study did not develop respiratory insufficiency or substantial changes in respiratory muscle strength, functional impairment, or forced vital capacity (FVC).[115,301] Difference between these clinically similar conditions is essential in providing accurate prognostic information to the patient and their family and is crucial for further treatment and management options.[9]

### Differential diagnosis

Lack of disease progression, an unusual patient history, or uncommon symptoms should prompt further investigation of the differential diagnosis of ALS [Table 1].[84,115,295]

### Common misdiagnosis of amyotrophic lateral sclerosis

Conditions that are commonly mistaken for or difficult to differentiate from ALS are multifocal motor neuropathy with conduction block, cervical spondylotic myelopathy, Kennedy disease (KD), and Post-polio syndrome (PPS).[64,115,257] Differentiating multifocal

**Table 1: ALS diagnosis: List of differential diagnosis and clinical overlap with ALS**

| | Differential diagnosis of ALS | Clinical overlap with ALS | Diagnostic to rule in/out |
|---|---|---|---|
| Hereditary conditions | Spino bulbar muscular atrophy (KD) | Progressive motor neuron degeneration[91] | Genetic testing. Identification of disease-specific mutations such as the CAG repeat expansion in the androgen receptor in SBMA[231] |
| | Hereditary spastic paraparesis | Gait disturbance, lower extremity spacicity[188] | Genetic testing[90] |
| | Acid maltase deficiency | Respiratory failure and muscle weakness are common clinical presentations[194] | Respiratory function tests, including the maximal static respiratory pressures, electromyographic examination and histochemical and biochemical studies of muscle biopsy specimens[250] |
| | Facioscapulohumeral muscular dystrophy | Muscle weakness | Presents initially with a distinct pattern of weakness involving the facial and scapular stabilizer muscles, with varying descending progression to involve the distal anterior leg or hip-girdle muscles. This is usually a benign dystrophy, but 20% of patients involved in certain clinical trials eventually become wheelchair-bound |
| | Adrenomyeloneuropathy | X-linked inherited metabolic disorder causing demyelination[241] | Detection of abnormal accumulation of very long chain fatty acids in plasma or red cells[212] |
| | Huntington disease | Progressive motor disturbances and involuntary movements[147] | Genetic testing[231] |
| | Hexosaminidase deficiency | Tremor, dystonia, spastic paresis, and psychosis have been noted in individual cases[134] | β-hexosaminidase subunits α and β assay, ganglioside GM-1 and GM-2 antibodies[115] |
| Metabolic conditions | Metal intoxication (especially iron and mercury) | Motor neuron dysfunction[155] | Heavy metals panel[207] |
| | Lathyrism | Pyramidal pattern of motor weakness, spasticity, and increased tone in the extensors and adductors of the thigh, as well in the gastrocnemius muscles with a "lurching gait"[175] | Diagnosed based on specified symptom criteria, and if clinically indicated[112] |
| | Organophosphate toxic effects | Peripheral neuropathy, fasiculations[214] | History of exposure to organophosphates |
| Immune and/ or inflammatory conditions | Multifocal motor neuropathy with conduction block | Peripheral nerve disorder characterized by progressive and asymmetric limb weakness, usually of the upper extremities. Minor sensory disturbances may be present | Nerve conduction studies of multifocal persistent partial conduction blocks on motor but not sensory nerves[218] |
| | Chronic inflammatory demyelinating polyneuropathy | Acquired neuropathy with highly variable clinical presentation[256] | Various electrodiagnostic criteria to include assessment of the distal compound muscle action potential duration[288] |
| | Myasthenia gravis | Various presentation of fatigable muscle weakness, especially of the legs and extra-occular movements | Presence of serum antibodies to acetylcholine receptor[309] |
| | Inclusion body myositis, polymyositis | Progressive muscle weakness and atrophy of the lower extremities | |
| | Multiple sclerosis | Episodic parasthesias and muscle weakness[15] | McDonald criteria, neuroimaging, spinal tap when clinically indicated[190] |
| Structural disorders | Cervical spondylotic mylopathy | Compression of the spinal cord that causes progressive neurologic deterioration[139] | Neuroradiologic imaging[86] |
| Neurodegenerative diseases | Corticobasal degeneration | Focal dystonia and myoclonia of the limbs, various clinical presentation and progression[99] | Current clinical diagnostic criteria under review[11] |
| | Multiple system atrophy | Sporadic neurodegenerative disorder with any combination of parkinsonian, autonomic, cerebellar, or pyramidal signs[321] | Indicated by cell loss, gliosis, and glial cytoplasmic inclusions in multiple CNS entities[321] |

*Contd...*

**Table 1: Contd...**

| | Differential diagnosis of ALS | Clinical overlap with ALS | Diagnostic to rule in/out |
|---|---|---|---|
| | Progressive supranuclear palsy | Extrapyramidal rigidity, bradykinesia, gait impairment, bulbar palsy, and dementia[82] | CT and MRI scans show midbrain atrophy early and later atrophy of the pontine and midbrain tegmentum and the frontal and temporal lobes. PET scans have shown frontal hypometabolism and loss of striatal D-2 dopamine receptors[82] |
| | Parkinson's disease | Progressive motor dysfunction and bradykinesia | Clinical presentation criteria based on stage of disease and response to various medications[37] |

PET: Positron emission tomograph, MRI: Magnetic resonance imaging, CT: Computed tomography, CNS: Central nervous system, SBMA: Spinobulbar muscular atrophy, KD: Kennedy disease, CAG: Cytosine-adenine-guanine

motor neuropathy from ALS is especially important, as patients with this neuropathy may benefit from intravenous immunoglobulin treatment, where ALS patients do not.[42,115] KD, also known as spinobulbar muscular atrophy, is an X-linked disorder associated with an expansion of trinucleotide repeats in the androgen receptor gene.[115,221] Significant features of this rare condition should prompt genetic testing for KD. This includes slow progressive LMN signs in the bulbar region and proximal limbs, absence of sensory nerve action potentials in nerve conduction studies, a family history without any male-to-male inheritance, gynecomastia, and hypogonadism.[148,221,327] Progression of KD is slower than that of typical ALS. Their life expectancy is unaltered, and patients usually do not develop any intellectual impairment.[327]

PPS presents with focal muscle weakness that very slowly progresses to other muscle groups over many years, and does not usually cause death.[257] Patients who present with chronic respiratory muscle weakness should have a thorough evaluation to rule out ALS, as the onset of these symptoms are found in about 3% of ALS patients.[235]

## Diagnostic tests

There is no single or absolute test for ALS, but an extensive workup is done to help rule out the various differential diagnosis. Table 2 illustrates a summary of different diagnostic tests for ALS.

### *Electrodiagnostic tests*

Electrodiagnostic studies are a useful diagnostic tool in the investigation of patients who may have ALS. EMG and nerve conduction studies are most sensitive to detecting the disease and can quantify its trademark characteristic of LMN degeneration.[57,65,115] This test can provide a baseline assessment of clinically unaffected areas. Typical EMG abnormalities in patients with ALS are fasciculation (fibrillation) potentials (FPs), and spontaneous denervation discharges, indicative of reinnervation.[35,203] Fibrillation potentials, which are characteristic of positive sharp waves visible on an EMG, may not manifest until one-third of the motor neurons has been lost. Their presence in clinically normal tissue can help facilitate early diagnosis.[295] FPs are also present in benign fasciculation syndrome (BFS), as well as many other conditions and can be highly complex in both ALS and BFS.[65,203] Multifocal distal triggering, axonal

**Table 2: Diagnostic tests for ALS**

| |
|---|
| Blood tests |
| Erythrocyte sedimentation rate |
| C-reactive protein |
| Hematological screen: Full blood count |
| Liver function tests: Alanine transaminase and aspartate transaminase levels |
| Creatine kinase |
| Creatine |
| Electrolytes: $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, $PO^4$ |
| Glucose |
| Lactate dehydrogenase |
| Thyroid function tests: Free tri-iodothyronine, free thyroxine, and thyroid stimulating hormone |
| Vitamins: B12, folate |
| Serum protein electrophoresis |
| Serum immunoelectrophoresis |
| β-hexosaminidase subunits α and β assay (where clinically indicated) |
| Ganglioside GM-1 antibodies (where clinically indicated) |
| Serum Borrelia titers and HIV tests (where clinically indicated) |
| Celiac serology (where clinically indicated) |
| Cerebrospinal fluid tests |
| Cell count |
| Protein |
| Glucose |
| Oligoclonal bands (where clinically indicated) |
| Neurophysiology |
| Nerve conduction velocities |
| Sensory and motor amplitudes |
| Presence of focal motor conduction block |
| Features of denervation on electromyography |
| Motor unit morphology |
| Imaging studies |
| MRI and/or CT (head and neck, thoracic, and lumbar) |
| Chest radiography |

CT: Computed tomography, ALS: Amyotrophic lateral sclerosis, MRI: Magnetic resonance imaging

conduction variability, and axonal conduction block are factors that lead to variable FP wave shape in ALS and BFS.[203] As ALS progresses, FP discharge rate increases and double same FPs become more prominent, implying that an axonal membrane abnormality has progressed.[203] Electrodiagnostic testing can be limited to confirming ALS in patients with very early signs of the disease due to the range of results produced from those who carry a clinical diagnosis of ALS.[18]

*Laboratory studies*

Typical labs drawn are erythrocyte sedimentation rate, serum and urine protein electrophoresis, thyroid function tests, serum calcium and phosphate measurements, and CSF analysis.[115] Heavy metal screening is indicated in patients with a potential history of exposure.[115] B-hexaminidase subunits alpha and beta activity should be tested in Ashkenazi Jews because deficiency in this enzyme mimics ALS, but in reality is the rare autosomal recessive genetic disorder, Tay-Sachs.[115]

*Neuroimaging*

Magnetic resonance imaging (MRI) studies of the brain and spinal cord are the most useful neuroimaging technique in ALS mainly to exclude syndromes that mimic ALS.[115] For example, new chromosome 9p-linked frontotemporal dementia (FTD)-ALS shows a distinct pattern of brain atrophy and neuropathological findings that can help differentiate from classical ALS.[33] Advanced neuroimaging technologies are useful research methods that may help identify specific ALS-associated pathologies in a noninvasive manner, but there are no specific features on an MRI that correlate well with ALS.[115] Neuroimaging is often done to help exclude differential diagnosis rather than confirming the diagnosis of ALS.

## TREATMENT

It has been suggested that there are shared environmental and genetic susceptibilities of several different neurological disorders, including PD, FTD, and ALS.[115,171,240] Clinical trials have been conducted giving the same treatment to patients with ALS, PD, and dementia. The assessments of these treatments could influence further diagnostic criteria of ALS.[115,240] Additionally, research has suggested a similarity in the etiology of both Down syndrome and SOD1-related ALS disease, due to their tau hyperphosphorylation.[122] Further understanding of how these mechanisms are connected may play a key role improving treatment and management for patients.

Development of treatments to alleviate ALS symptoms is the foundation for providing proper healthcare to patients. In Table 3, we summarized the current therapeutic agents that have shown promising results in preclinical assessment and some of them have already gone through clinical trials. These compounds were grouped based on the pathophysiological model of the disease.

Riluzole is currently the only FDA-approved drug treatment identified to have beneficial use in the survival of patients with ALS. Two clinical trials demonstrated evidence of increased survival for the riluzole-treatment group compared with controls.[19,20,162,247] There is some debate on riluzole's precise mechanism, as three mechanisms in decreasing the neuro-toxic effect of glutamate have been recognized. Riluzole is known to trigger presynaptic inhibition and subsequent release of glutamate from cerebrocortical nerve terminals.[316] It inactivates voltage-gated sodium channels and is a noncompetitive NMDA receptor antagonist.[71]

The recommendation dose of riluzole is 50 mg twice daily for patients with definite or probable ALS for duration <5 years, an FVC >60%, and no tracheostomy.[201] Other than riluzole, no other new treatment has been identified that be able to increase the ALS life expectancy.[199] Palliative care can help with the management of ALS symptoms and improving the quality of life. A summary of available palliative care for different symptoms of ALS is provided in Table 4.

## AMYOTROPHIC LATERAL SCLEROSIS MANAGEMENT

Over the past two decades, the management of ALS has changed considerably. Although still incurable, ALS is not untreatable. Emphasis has been made in treatments and interventions that prolong survival.[43,164,204,227,243] While there are no medications that halt or reverse the progressive loss of neurons, importance has been given to management strategies that optimize the quality of life and help maintain the patient's autonomy for as long as possible.[164,270,300,323] Coordinated multidisciplinary care from neurologists, physical therapists, speech therapists, occupational therapists, respiratory therapists, social workers, dietitians, and nursing care managers should be considered for managing patients with ALS to enhance health care delivery, prolong survival, and the quality of life.[201,207,300] Important issues should be discussed with patients and relatives as soon as they are willing to, such as concerns that might arise about the course of the disease, the nutritional and respiratory management during late stages of life, and the patients advanced directives and end-of-life decisions.[270,323]

### Multidisciplinary management

In recent years, multidisciplinary ALS treatment facilities have emerged as a result to a shift in the approach of health care delivery to ALS patients. By treating only ALS patients, these multidisciplinary ALS clinics gather

**Table 3: Compounds tested for ALS treatment**

| Pathophysiological category | List of compounds tested for ALS |
|---|---|
| Antiapoptotic | |
| Mitochondrial impairment and aberrant calcium handling are two major components of motor neuron injury that lead to activation of the apoptotic cascade[58,141] | Dexpramipexole (R−(+) pramipexole)[45]<br>Minocycline[104]<br>Pentoxifylline[192]<br>Omigapil (TCH-346)[200]<br>Caspase family inhibitor (fluoromethylketone) (zVAD-fmk)[166] |
| Anti-inflammatory | |
| Reactive astrocytes and microglia as well as infiltrating T lymphocytes and macrophage were found to have a main role in neurodegenerative process and neuroinflammation in ALS patients[248,296] | AM-1241 (aminoalkylindole family)[268]<br>Celastrol[143]<br>Celecoxib[104]<br>EPO[163]<br>Glatiramer acetate[193]<br>Minocycline[105]<br>Nordihydroguaiaretic acid[32]<br>Arundic acid (ONO-2506)[68]<br>Pioglitazone[144]<br>RO-28-2653 (synthetic inhibitor of MMPs)[172]<br>Rofecoxib[13]<br>Thalidomide[282] |
| Anti-excitotoxicitory/antiglutamatergic | |
| Excitotoxicity is mainly modulated by the release of glutamate. ALS patients have a decreased glutamate transport capacity due to loss of EAAT2 transporter receptors. This lead to increase of glutamate levels in the CSF of ALS patients[167,297] | Ceftriaxone[294]<br>Cobalamin[137]<br>Gabapentin[320]<br>Lamotrigine[253]<br>L-Arginine[128]<br>Memantine[57]<br>N-acetylated alpha-linked acidic dipeptidase[98]<br>Riluzole[20]<br>Talampanel[232]<br>Nordihydroguaiaretic acid[32]<br>Glatiramer acetate[193] |
| Antioxidant | |
| In ALS mutations of the SOD gene reduce its superoxide dismutase activity therefore leading to elevation of free radical accumulation and oxidative stressc.[267,272,277] Several antioxidant compounds have been found to protect neurons | AeOL-10150 (Aeolus)[226]<br>Ammonium tetrathiomolybdate[290]<br>Celastrol[143]<br>Creatine[108,186]<br>Coenzyme Q10[89]<br>Edavarone[328]<br>N-acetylcysteine[174]<br>Olesoxime (TrO19622)[29,30,181]<br>R(+) pramipexole[45]<br>Tamoxifen[36]<br>Tocopherol (Vitamin E)[74] |
| Anti-aggregation | |
| Mutation in SOD1 gene causes conformational instability of the encoded protein leading to the formation of aggregates. Cellular proteins aggregation, such as the Bunina bodies, is a well-known feature of ALS.[94] Preventing these cellular aggregates can increase the survival of motor neurons | Ariclomol[61]<br>Scriptaid[55]<br>Sodium phenylbutyrate[60]<br>Valproate[237]<br>Celastrol[143] |
| Neuroprotective and neurotrophic growth factor | |

*Contd...*

**Table 3: Contd...**

| Pathophysiological category | List of compounds tested for ALS |
|---|---|
| Several mechanisms such as glutamate excitotoxicity, aberrant protein aggregation, and oxidative stress lead to neurodegeneration, either loss or shrinkage of neurons, in ALS[88,94,125] | BDNF[220] |
| | Ciliary neurotrophic factor[202] |
| | GDNF[152,284] |
| Neuroprotective drugs can help with slowing down the neuronal damage. These growth factors stimulate the growth of new neurons (neurogenesis) and the repair the damaged ones[119,152] | r-IGF-1[278] |
| | Xaliproden[161] |
| | VEGF[292] |
| | EPO[40] |
| | rh-GSF[215] |
| | rh-HGF[135] |
| | Rasagiline[314] |

ALS: Amyotrophic lateral sclerosis, rh-HGF: Recombinant human hepatocyte growth factor, rh-GSF: Recombinant human granulocyte-stimulating factor, VEGF: Vascular endothelial growth factor, r-IGF-1: Recombinant protein, insulin like growth factor-1, GDNF: Glial cell-derived neurotrophic factor, BDNF: Brain-derived neurotrophic factor, SOD1: Superoxide dismutase 1, CSF: Cerebrospinal fluid, MMPS: Matrix metalloproteinases, EPO: Erythropoietin, EAAT2: Excitatory amino acid transporter 2

great resources and clinical expertise that can facilitate the management and provide optimized care of this progressive disease.[84,293] Although data are limited, some studies, but not all, have suggested that multidisciplinary ALS clinics have improved the quality of life and lengthened survival compared to ALS patients in general neurology clinics.[27,50,84,201,293,300,332]

These multidisciplinary ALS specialized clinics can better assist in managing the complex issues associated with ALS, such as psychosocial problems, nutrition, dysphagia, dysarthria, functional decline, and respiratory symptoms. Both the American Academy of Neurology (AAN) and the European Federation of Neurological Societies recommended that after diagnosis, the patient and caregivers should be referred to a multidisciplinary clinic and receive regular support from a multidisciplinary care team to optimize health care delivery and prolong survival.[84,201]

### Respiratory management

The most common cause of death in ALS is due to respiratory failure with or without pneumonia. The presenting symptoms of respiratory muscle weakness, secondary to progressive motor neuron degeneration, result in reduced ventilation.[84,110,323] These symptoms may include dyspnea on exertion or talking, orthopnea, disturbed sleep, excessive daytime somnolence, morning headaches, fatigue, anorexia, depression, poor concentration, vivid nightmares, and nocturia.

Since ALS mortality is mostly caused by respiratory failure, the assessment and management of respiratory function are of great importance. The most common and widely available measure for detecting respiratory decline is the examination of the patient's FVC.[59,92,110] Shorter survival is associated with lower FVC.[62] Another alternative is the Sniff nasal inspiratory pressure test, which acts as a good measure of diaphragmatic strength and had a better predictive value than FVC.[92,176,281] The current guidelines given by the AAN suggest that noninvasive ventilation (NIV) should be considered to treat respiratory insufficiency.[110,176,201] Therapeutic use of NIV is thought to improve survival, slow the decline of FVC, and improve the quality of life in ALS patients.[9,84,110,300]

### Nutritional management

Most ALS patients develop dysphagia which leads to malnutrition and weight loss. The consequences of this progressive deterioration include restricting ample nutrition, dehydration, choking, aspiration, and weight loss. As a consequence of the dysphagia present in these patients, the risk of insufficient caloric and fluid intake increases, leading to worsening of weakness and fatigue.[26,51,183,201] Through the use of video fluoroscopic evaluation, it is possible to detect which food consistencies are better handled by the patient. Nutritional management consists initially in altering food consistency, but eventually percutaneous endoscopic gastrostomy (PEG) or similar device may be needed for enteral feeding.[140] Most guidelines recommend that supplementary enteral feeding should be considered in patients whose body weight falls more than 10% of their prediagnostic weight.[84,164] PEG is the standard procedure for enteral feeding and has been found to be helpful in stabilizing weight loss common in ALS.[165,183] However, there is not enough data to refute or support a specific timing of PEG insertion in ALS patients.[140,201,323] Furthermore, there are limited data that correlates prolonged survival with PEG placement and the impact of PEG on the quality of life in ALS patients.[187,201,300] It is suggested that nutritional supplementation using PEG should be done before FVC falls below 50% of predicted values because of the increasing mortality risk of the procedure as respiratory function declines.[164,183,187,201]

## DIETARY SUPPLEMENTATION

### Vitamin E and Vitamin A

Although the pathophysiologic causes of ALS are not clearly understood, it is hypothesized that free radical stress is a main component of the cell degeneration

**Table 4: Palliative care for ALS symptoms**

| Symptoms | Treatment |
|---|---|
| Disability and weakness | Orthotics (ankle foot orthosis, neck collars)<br>Physiotherapy<br>Adaptive aids (walking frame, wheelchair) |
| Dysphagia | Assessment by speech therapist and dietitian<br>Safe swallowing techniques and modified diet<br>Insertion of gastrostomy tube dyspnea and poor cough<br>Ventilator support<br>Morphine or benzodiazepines<br>Chest physiotherapy<br>Suction machine<br>Manually assisted coughing techniques |
| Pain (i.e., musculoskeletal pain and cramps, fasciculations and spasticity, skin pressure pain caused by immobility) | Physiotherapy, NSAIDs<br>Muscle relaxants (baclofen, botulinum toxin)<br>Anticonvulsants (gabapentin)<br>Re-positioning and pressure area care<br>Opioid drugs<br>Pressure-relieving cushions and mattress |
| Dysarthria | Assessment by speech pathologist<br>Communication aids<br>Educate family and caregivers<br>Cognitive changes (frontal lobe dysfunction or dementia)<br>Explain symptomatology to caregivers and family<br>Antidepressant therapies |
| Sialorrhea | Anticholinergic antidepressants (amitriptyline)<br>Anticholinergic drugs (glycopyrronium bromide)<br>Botulin toxin injections<br>Radiation of salivary glands<br>Mouth care products<br>Suction |
| Thickened saliva | Natural remedies (papaya)<br>Ensure adequate hydration<br>Saline nebulisers; nebulised N-acetylcysteine<br>Suctioning of the mouth<br>Mouth care |
| Emotional lability | Educate patients with ALS and caregivers<br>Amitriptyline<br>Benzodiazepines<br>Dextromethorphan hydrobromide/quinidine sulfate |
| Depression and anxiety | Counseling<br>Benzodiazepines<br>Antidepressants |
| Sleep disturbance | Treat underlying problem<br>Respiratory review, noninvasive ventilation<br>Benzodiazepines, tricyclic antidepressants |
| Constipation | Dietary changes (increase fluid and fiber intake)<br>Use formulations high in bran, bulk, or fiber<br>Regular oral aperients (Movicol or suppositories) |

ALS: Amyotrophic lateral sclerosis, NSAIDs: Nonsteroidal anti-inflammatory drugs

contributing to ALS progression and onset.[273] Since Vitamin E or α-tocopherol functions as an antioxidant in neural cell membranes, there have been several studies testing its role in ALS.[74,106,197,224,225,227] In a research study by Gurney *et al.* using transgenic mice, Vitamin E supplements delayed the onset and slowed the course of ALS but did not affect survival rates.[111] When a similar study was applied to humans, Vitamin E intake only slowed the progression of the disease.[74,107,225] Michal Freedman *et al.* also showed that higher than normal levels of serum Vitamin E was associated with reduced risk of ALS and a small protective effect of Vitamin E supplements is present in patients with lower than normal Vitamin E levels.[197]

In other studies, the efficacy of Vitamin A (beta-carotene) supplementation was investigated among ALS patients. Their results showed that beta-carotene neither has any neuroprotective effect on ALS patients nor helps with slowing down the progression of the disease.[197,209]

### Creatine

An investigational study by Klivenyi *et al.* on transgenic mouse with ALS showed a possible protective effect of dietary creatine supplementation on neurons.[149] Their result presented improved motor performance and extending survival of transgenic mice.[149] A follow-up study by Andreassen *et al.* explained how creatine intake may improve cellular glutamate transporter, an effect that would prevent a glutamate excitotoxicity, a proposed mechanism of ALS.[10] Clinical trials on human have shown, however, that dietary creatine supplementation did not have an impact on the survival rate of ALS patients or slowing disease progression.[108]

### Pu-erh tea extract

A recent research study from Jilin University in China suggests that pu-erh tea extract (PTE) can help in preventing the rapid advancement of ALS in patients. The results of the study suggest that PTE can posttranscriptionally prevent the progression of FET family proteins that are associated with ALS. Also, results from the study suggest that PTE induces FUS/TLS protein degradation via lysosome-dependent pathway. With long-term intake, PTE may prevent protein aggregation and enable cells to maintain function within normal levels of protein. Further studies are required to ascertain the efficacy of PTE on FET *in vivo*.[329]

## SURVIVAL AND PROGNOSIS

ALS is a progressive condition in which more than half of patients diagnosed do not survive within the first 30 months after symptom onset. Only 20% of the patients survive between 5 and 10 years after symptoms onset.[285] Reduced survival to the disease is related to the older age of symptom onset, early respiratory muscle dysfunction, and bulbar onset disease. On the other hand, limb-onset disease, younger age at presentation of the disease and longer diagnostic delay are independent predictors of prolonged survival.[312]

Some ALS subtypes vary according to prognosis. LMN form of ALS, which includes flail-limb variant and PMA, shows a slower progression than other forms of ALS.[285,312] A prognosis of 2–4 years is seen in the pure bulbar palsy phenotype, which usually affects women older than 65 years of age. In this type of ALS, the disease remains localized to the oropharyngeal musculature and UMN features predominate.[285]

## CONCLUSION

This study covered a broad range of information about ALS from epidemiology to molecular mechanism and treatment of the disease. Unfortunately, ALS is considered an incurable disease, with an expected life expectancy of 3–5 years after the onset of symptoms.[115] Although there are many antioxidants and supplements that have been proposed as an alternative treatment for ALS, most of them have not been verified in research studies or studies performed lack validity or substantial proof in their methodology.[225] It is important to continue nutritional studies in order to provide better care to ALS patients, as some evidence has shown they may help to alleviate the impact of the disease on their daily lives. For instance, a coherent and in-depth research on alpha-tocopherol and creatine is needed to confirm the known findings on these supplements.

There have been important advancements in the understanding of ALS pathophysiology. Nineteen genes and genetic loci have been found that are associated with ALS.[4] Identifying the molecular pathways underlying ALS will provide the insight to therapeutic approaches. There are currently several clinical trials in place for drugs that are antiapoptotic, anti-aggregation, antioxidant, anti-excitotoxicitory, anti-inflammatory, neuroprotective, and neurotrophic growth factor.[333] Current discoveries of the underlying mechanism of ALS have helped to slow down the progression of the disease. Thus, the future treatments should aim toward preventing neuronal damage, as patients progress from their initial onset.

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# Riluzole and amyotrophic lateral sclerosis survival: a population-based study in southern Italy

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Riluzole is to date the only treatment that prolongs amyotrophic lateral sclerosis (ALS) survival. However, results on the efficacy of riluzole in observational population-based studies with a longer follow-up are conflicting and it is still unclear if the effect of the drug is limited to an early stage of the disease and to some specific subgroups of patients. The objective is: (i) to evaluate the effect of riluzole on ALS survival in a cohort of incident cases; (ii) to examine whether bulbar-ALS benefits from the medication to a greater extent and (iii) to assess the efficacy of the drug in elderly patients. Source of the study was a prospective population-based registry of ALS established in Puglia, Southern Italy. We examined survival of 126/130 incident ALS cases diagnosed during the period 1998–1999. Seventy-three patients were prescribed riluzole and the remaining 53 were not. Riluzole therapy increased survival rates at 12 months by approximately 10% and prolonged survival by 6 months (18.2 months vs. 12.4; peto-test: 2.78; $P = 0.09$). This beneficial effect was present amongst bulbar-onset ALS (peto-test: 4.11; $P = 0.042$), but not in subjects with limb-onset (peto-test: 0.48; $P = 0.4$). In patients aged $>70$ years riluzole treatment was associated with an 8 months longer median survival time [15.4 months vs. 7.1] and a reduction in mortality rate at 12 months by 27%, regardless of site of symptoms onset. In multivariate analysis, riluzole use was an independent predictor of survival at 12 months from the diagnosis with borderline significance ($P = 0.06$). Riluzole was effective amongst cases with bulbar-onset ALS ($P = 0.04$), whereas in subjects with limb-onset there was no effect on survival at 12 months ($P = 0.5$). In each model riluzole did not influence survival at 24 months. Conversely, riluzole use was associated with an improvement in survival amongst elderly patients both at 12 ($P = 0.07$), at 24 months ($P = 0.03$) and in the entire follow-up period ($P < 0.04$). In this population-based series, we found that riluzole therapy improves ALS survival. The efficacy of the drug was present amongst bulbar-onset ALS and older patients, but not in subjects with limb-onset. The favourable effect of the drug was transient, as it was lost in prolonged follow-up. Our observations support the use of riluzole at an early stage of ALS in bulbar and elderly patients. However, the appropriate duration of riluzole treatment remains to be established.

## Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative motor-neuron disorder of unknown origin, with no effective cure; the progression is rapidly progressive, leading to death within 3–5 years [1]. Riluzole is currently the only drug capable to improve survival of ALS patients, as evidenced by two randomized trials. 2,3].

Several issues regarding riluzole remain undetermined. First, is riluzole truly effective in increasing survival of ALS patients? Data from two population-based studies of ALS in Europe showed that riluzole was an independent predictor of survival [4] and that riluzole prolonged survival by at least 4 months [5]. These findings are in contrast to two other longitudinal population-based studies that showed a trend towards shorter or unchanged survival in the last decade, despite the introduction of riluzole, percutaneous endoscopic

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gastrostomy (PEG) and non-invasive ventilation (NIV) [6,7].

The second issue surrounding riluzole is the appropriate duration of the treatment. Two studies [5,8] have shown that the drug is ineffective in advanced ALS, suggesting that the medication should be started as early as possible and discontinued in the late stages of disease. This is in direct contrast to the AAN guidelines for riluzole [9] which state that the medication should only be prescribed for patients with probable or definite ALS by El Escorial criteria (EEC).

Finally, it is unclear whether specific subgroups of ALS patients benefit more from riluzole administration. A greater efficacy of riluzole on patients with bulbar-ALS was noted in the first clinical trial of riluzole ($n$ = 155) [2,5], but this finding was not replicated in the larger subsequent placebo-controlled trial ($n$ = 959) [3]. Interestingly, a recent observational study again found that riluzole was particularly effective in bulbar-onset patients, prolonging survival [5]. The effectiveness of riluzole in older patients is not well defined. Patients 75 years and older have been included in only one randomized clinical trial [8] which did not detect any difference in survival between the treatment groups.

The aim of the present study was three fold: (i) to evaluate the effect of riluzole on survival in a cohort of ALS incident cases from a population-based study conducted in Southern Italy and to eventually determine the appropriate duration of treatment, (ii) to examine whether riluzole is more effective in bulbar ALS and (iii) to assess if the drug exerts beneficial effects in elderly patients.

## Material and methods

A prospective ALS registry, established in Puglia, Southern Italy, in 1997, was the source of cases for this study [10]. The surveillance began on January 1, 1998. The registry has several sources of information that have been described elsewhere [10].

The diagnosis of ALS was based on EEC [11] and their Airlie-House revised version of 1998 (AHC) [12]. Riluzole is provided free of charge to Italian ALS patients provided it is prescribed by a neurologist working within the National Health system. All patients with ALS diagnosis are eligible for riluzole treatment in Italy.

Using this multisource registry, we identified all newly diagnosed ALS cases resident in Puglia in the 2-year period 1998–1999. All cases were routinely followed-up during the course of their illness on average every 6 months by direct examination or by telephone. Data was collected on the medications and treatments provided to patients (e.g. riluzole, NIPV, PEG, tracheostomy). Date and cause of death was recorded and death certificates were obtained from the National Death Data Base Registry. Date of last follow-up for this study was 30 June 2004. Death status was checked at censoring date for all patients in the study.

### Statistical analysis

All patients gave informed consent to participation in the study; data were stored in a centralized database with separate anonymous files. Comparison of demographic features between cohorts employed either Mann–Whitney, $t$-test or chi-squared test. Survival curves were estimated by Kaplan–Meier method and differences in survival were measured by Peto and log-rank tests. Survival interval is from time of diagnosis. Peto test was used to emphasize the information on differences at the beginning of survival curves [13]. Multivariate analyses of the risk for death associated with selected independent variables were performed using the Cox proportional hazard model. An intention to treat analysis was employed.

## Results

During the 2-year study-period we identified 130 patients (81 males, 49 females); data concerning riluzole prescription were missing for four patients (3%). Of the remaining 126 patients, 73 (58%) were prescribed riluzole on at least one occasion and 53 (42%) did not receive riluzole at any stage of the illness. The choice for treatment was made by the neurologist member of the registry, with informed consent by the patient. No patient refused the treatment. In addition, no treatment withdrawal was referred during the entire follow-up period.

Demographic and clinical variables were similar amongst patients receiving and not receiving riluzole (Table 1). Riluzole could have been prescribed based on progression of the disease. We measured progression of the disease with the onset-diagnosis interval (ODI), that were similar in the two groups. (Fig. 1; chi square, 2.4; $P = 0.6$). Only a small percentage of our patients underwent PEG (6%) or NIV (2.5%; Table 1). PEG was generally initiated after 26 months in this group, much later than riluzole prescription.

### Univariate analysis

Median survival time from diagnosis was 5.8 months longer amongst patients prescribed riluzole compared with patients that did not receive riluzole (18.2 months vs. 12.4). Riluzole administration reduced mortality rate at 6 and 12 months by 8.3% (6.8% vs. 15.1%) and 11.6% (20.5% vs. 32.1%; Peto, 2.78; $P = 0.09$; log-rank, 0.08; $P = 0.78$) respectively. At 18 months from

**Table 1** Clinical features at diagnosis of riluzole and non-riluzole cohorts

| Variable | Patients prescribed Riluzole ($n$ = 73) | Patients not prescribed Riluzole ($n$ = 53) |
|---|---|---|
| Median Age (range) | 64.3 years (32–80.2) | 66 years (19–80)<br>Mann–Whitney, 0.002; $P$ = 0.9 |
| Gender (M/F) | 43/30 | 35/18<br>Chi-square, 0.4; $P$ = 0.5 |
| Bulbar-onset | 20 (27%) | 13 (25%)<br>Chi-square, 0.02; $P$ = 0.9 |
| Spinal-onset | 53 (73%) | 40 (75%)<br>Chi-square, 0.02; $P$ = 0.9 |
| Time to diagnosis (range) | 8.6 months (1–70.7) | 10 months (1.2–52)<br>$t$-test, 0.5; $P$ = 0.6 |
| Median survival time from the diagnosis (range) | 18.3 months (1.8–48) | 12.4 months (0.3–50)<br>$t$-test, 1.4; $P$ = 0.2 |
| Possible + suspected ALS | 31 (42%) | 23 (43%)<br>Chi-square, 0.06; $P$ = 0.9 |
| Probable + definite ALS | 42 (58%) | 30 (57%)<br>Chi-square, 0.06; $P$ = 0.9 |
| NIV | 3 (4%) | 0<br>Chi-square, 0.8; $P$ = 0.1 |
| PEG | 7 (10%) | 1 (2%)<br>Chi-square, 1.9; $P$ = 0.08 |

NIV, non-invasive ventilation; PEG, percutaneous endoscopic gastrostomy; ALS, amyotrophic lateral sclerosis.

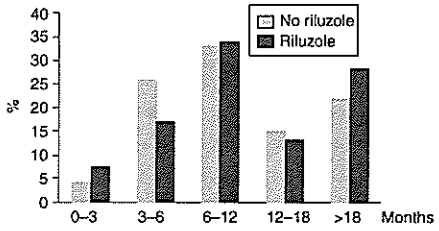


**Figure 1** Histogram of onset–diagnosis interval distribution between amyotrophic lateral sclerosis patients treated with riluzole and untreated patients in Puglia ($n$ = 126; chi-square, 2.4; P = 0.6).

the diagnosis mortality rate was 4.6% lower amongst the treated group. After the 18 month time point, there was no difference in mortality rates between the two groups (Fig. 2).

Patients with bulbar-onset disease ($n$ = 33) benefited more from riluzole administration than patients whose symptoms started in the limbs. Riluzole prescription was associated with an 8 months longer median survival time amongst bulbar-onset ALS [17.1 months (range: 3.7–36.7) compared with 9.2 months (2.9–28.5)]. Mortality rates at 6 and 12 months were 5% (1/20) and 25% (5/20) in bulbar-onset ALS patients receiving riluzole, compared with the 31% (4/13; peto-test:4.11; $P$ = 0.04; log-rank test, 1.1; $P$ = 0.29) and 54% (7/13) of the non-treated group. This effect was lost after 18 months of follow-up (Fig. 2). There was no difference in median survival amongst limb-onset ALS patients receiving and not receiving riluzole. Demographics and clinical characteristics were similar amongst bulbar-onset receiving and not receiving riluzole (data not shown).

Riluzole had a beneficial effect on prognosis amongst Italian ALS patients aged > 70 years ($n$ = 34). Median survival was prolonged by 8.3 months (15.4 months vs. 7.1 months) and 12 month mortality rate was slightly but not significantly decreased [57% (8/14) vs. 30% (6/20); peto = 0.33; $P$ = 0.5; log-rank, 0.78; $P$ = 0.33]. The beneficial effect of riluzole was present in elderly patients regardless of site of symptom onset. In patients who took riluzole this favourable effect was evidenced even when considering only limb-onset cases. Median survival amongst limb-onset cases >70 years taking riluzole was 18 months (1.8–22.6) compared with 8 months (1–12.5) amongst the same demographic not taking riluzole.

Riluzole did not have a beneficial effect on prognosis amongst patients with a rapidly progressive disease course or with a limited spread onset of the disease. Amongst the rapidly progressive subgroup (whose ODI was ≤ 6 months; $n$ = 91), there was no difference in

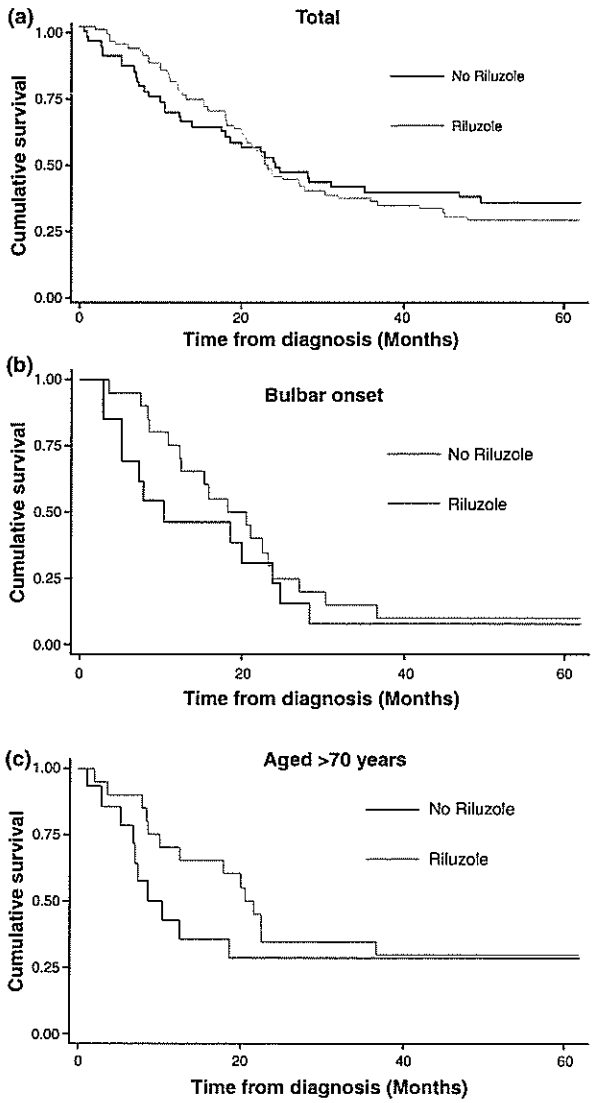


**Figure 2** Kaplan–Meier survival curves from diagnosis for amyotrophic lateral sclerosis cases diagnosed in Puglia in 1998–1999, stratified according to riluzole use. Subtitles for the three graphs are as follows: (a) entire case series ($n = 126$; log-rank: 0.08; $P = 0.78$; peto: 2.78; $P = 0.09$); (b) bulbar onset cases ($n = 33$; log-rank: 1.1; $P = 0.29$; peto: 4.11; $P = 0.04$); (c) patients aged >70 ($n = 34$; log-rank: 0.78; $P = 0.37$; peto: 0.33; $P = 0.5$).

survival between patients treated and not-treated with riluzole [20.5 months (1.8–32; $n = 22$) vs. 18.8 (14–31; $n = 13$; log-rank, 0.07; $P = 0.8$)]. Finally, in patients with suspect and possible ALS by EEC ($n = 54$) survival rates at 12 months (78.1% vs. 73.9%) and median survival time were similar in the two groups (18.2 months vs. 16.3; log-rank, 1.5; $P = 0.2$).

Finally, as patients prescribed riluzole had more interventions, we examined the contribution on survival of more active interventions by ALS multidisciplinary clinics in our territory area. Despite a higher percentage of patients prescribed riluzole in the multidisciplinary clinics cohort than in the general neurology cohort (66% vs. 43%), we found no difference in median survival times of ALS patients attending ALS multidisciplinary clinics ($n = 84$) compared with those attending general neurology clinics ($n = 42$; 17.6 vs. 18 months; log-rank = 0.11; $P = 0.76$).

### Multivariate analysis

Cox proportional model showed that riluzole use was a predictor of favourable survival at 12 months from the diagnosis in the entire case series with slight significance (HR: 0.51; 95%CI: 0.25–1.03; $P = 0.06$), after adjustment for age, gender, site of symptoms onset and ODI. This effect was stronger amongst bulbar-onset ALS, after adjustment for age and ODI (HR: 0.26; 95%CI: 0.07–0.92; $P = 0.04$); conversely, subjects with limb-onset treated with riluzole did not present favourable effects on survival at 12 months (HR adjusted for age and ODI: 0.72; 95%CI: 0.30–1.74; $P = 0.5$). In each model the positive effect of riluzole on survival was lost after 24 months.

Multivariate model revealed that riluzole use was associated with an improvement in survival even amongst elderly patients after adjustment for age, gender, site of onset and ODI both at 12 months (HR: 0.33; 95%CI: 0.1–1.07; $P = 0.07$), at 24 months (HR: 0.36; 95%CI: 0.1–0.92; $P = 0.03$) and in the entire follow-up period (HR: 0.36; 95%CI: 0.1–0.93; $P < 0.04$).

## Discussion

In this population-based incident study riluzole treatment was associated with a 10% reduction in mortality at 1 year, corresponding to an increase in survival of 6 months. The beneficial effect of the drug was transient, as it was lost after 24 months of follow-up.

Although we did not find differences in survival with log-rank test, a trend towards improvement in survival was detected with peto-test, which emphasizes early survival. This observation is in contrast to a previous retrospecitve study [14], but consistent with the result of the Irish study [5] and may be related to the transient effect of the drug. In the retrospective clinical-based study [14], a stronger effect of riluzole was found but the subjects in the study were younger and the median survival time was longer (approximately 40 months) than in our cohort.

The improvement in ALS survival was most marked amongst patients with bulbar-onset of symptoms (8 months), whereas no significant effect was present

amongst patients with limb-onset. We found that riluzole administration is effective in prolonging survival amongst older patients, regardless of site of symptom onset. This is, to our knowledge, the first observational study to show an effect of riluzole amongst the elderly.

The ability of retrospective observational studies, such as the current study, in assessing drug efficacy is limited compared with double-blind, placebo-controlled clinical trials. The most important limitation of an observational study is the lack of control for unknown prognostic factors that can be differentially distributed in the treatment and non-treatment cohorts [15,16]; however, imbalances between treatment groups for important risk factors as age are not infrequent even in placebo-controlled double-blind trials [8]. Moreover, in our study, the two groups were similar in all measured clinical and demographic characteristics with prognostic value (age at onset and diagnosis, gender, site of symptom onset, ODI, classification according to EEC and AHC).

As in other studies [15], we had no data on vital capacity (VC); however the role of VC as prognostic indicator remains uncertain; some studies [17] found that VC at baseline is a predictor of survival, whilst others [18] did not. VC has some limitations as a measure for predicting respiratory failure in clinical practice, especially in bulbar-ALS and cases with more severe illness [19].

Furthermore, no differences were found in median survival times of patients who attended ALS multidisciplinary clinics compared with patients followed-up by general neurology clinics in this area only few patients underwent PEG or NIV and only in the latest stage of their illness. Finally, a placebo effect cannot be excluded, as both the patients and the physicians were unblinded. However, this seems implausible because survival was used as a measure of treatment efficacy.

The main strength of the population-based observational studies is that they are characterized by a broader range of clinical phenotypes compared with the selected subjects included in clinical trials in ALS tertiary centres. The findings are more probably to be representative of the drug's effectiveness in every day clinical practice, as subjects are more probably to reflect the management of ALS. Moreover, clinical trials are characterized by a short period of follow-up (18 months) compared with observational studies (5 years). This aspect of study design is important in the case of riluzole, as the beneficial effects of the medication appear to be lost after 18 months. Consistent with this hypothesis is a recent study carried out in a sample of long survival ALS (more than 10 years) from the King's Database [18] that found that only a few of the ALS long survivors received any interventions.

Our results of a favourable but transient effect of riluzole on ALS survival are similar to placebo-controlled trials [1,2] and to a population-based study in Ireland [5]. The lack of effect in the later stage of the disease (after 18 months) in our study is also consistent with the negative results of a randomized clinical trial carried out in ALS patients with advanced disease [8]. A study on transgenic rats demonstrated that the deficit in glutamate uptake becomes more severe by end-stage of the disease and is probably to be the cause for the loss of efficacy of the drug in advanced ALS [20].

In our case series, patients with bulbar-onset ALS benefit more from riluzole than patients with limb-onset disease. This observation has been previously reported [2,5]. and has been related to the shorter ODI of bulbar-ALS [4,5] and the earlier start of the drug, when the spread of motorneuron degeneration is limited; however, when we looked at cases characterized by a short ODI ($\leq$ 6 months) and limited spread of signs (restricting the analysis to possible and suspected cases), we did not find difference in survival, suggesting that neither of these two factors could explain the selective benefit of riluzole for bulbar-ALS; confirming these data, multivariate analysis revealed that the effect of riluzole was independent of ODI. An overall difference in glutamate uptake in different areas of the brain could explain the better efficacy of riluzole in bulbar-onset cases, characterized by less extensive deficit of glutamate transport capacity, compared with spinalonset cases [20].

Our study demonstrated that riluzole administration in patients > 70 years associated with a 30% increase in survival at 12 months and an 8 months longer survival, regardless of site of symptoms onset. Despite the lack of significant effect of the drug on survival on univariate analysis, we observed a favourable effect of the drug on survival on multivariate analysis, after removing a possible confounding effect of gender, site of symptoms onset and ODI. These results indicate that riluzole exerts beneficial effects in older ALS patients.

The main limitation of our study, as in most ALS population-based studies, was the limited sample size that could have hampered the power of our analysis in some subgroups. Finally, we analysed the data with the intention to treat approach whilst compliance and duration of treatment were not considered.

In conclusion, even if randomized clinical trial is the unique gold standard for the evaluation of treatment, observational cohort studies like ours can give additional information about the use of riluzole in clinical practice. Our study supports the use of riluzole in the early stages of ALS because it improves surviv-

orship for a limited period of time. Bulbar-onset cases and elderly patients both experience significant benefits from therapy. Further studies are needed to establish if the interruption of riluzole should eventually be considered 2 years after the diagnosis.

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## Appendix: Sclerosi Laterale Amiotrofica-Puglia (SLAP Registry)

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# REFERENCE #10

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State of Hawaii
Department of Health

Letter of support to add amyotrophic lateral sclerosis (ALS) as a debilitating condition for which the use of cannabis is appropriate.

To whom it may concern:

Significant advances have increased our understanding of the molecular mechanisms of amyotrophic lateral sclerosis (ALS), yet this has not translated into any greatly effective therapies. It appears that a number of abnormal physiological processes occur simultaneously in this devastating disease. Ideally, a multidrug regimen, including glutamate antagonists, antioxidants, a centrally acting anti-inflammatory agent, microglial cell modulators (including tumor necrosis factor alpha [TNF-a] inhibitors), an anti-apoptotic agent, one or more neurotrophic growth factors, and a mitochondrial function-enhancing agent would be required to comprehensively address the known pathophysiology of ALS.

Remarkably, cannabis appears to have activity in all of those areas. Preclinical data indicate that cannabis has powerful anti-oxidative, anti-inflammatory, and neuroprotective effects. In the G93ASOD1 ALS mouse, this has translated to prolonged neuronal cell survival, delayed onset, and slower progression of the disease.

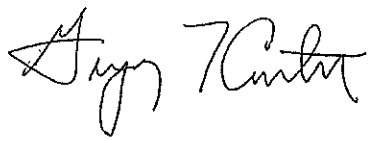
Cannabis also has properties applicable to symptom management of ALS, including analgesia, muscle relaxation, bronchodilation, saliva reduction, appetite stimulation, and sleep induction. With respect to the treatment of ALS, from both a disease modifying and symptom management viewpoint, clinical trials with cannabis are the next logical step. Based on the currently available scientific data, it is reasonable to think that cannabis might significantly slow the progression of ALS, potentially extending life expectancy and substantially reducing the overall burden of the disease.

Peer reviewed references in the medical literature are provided for all of these statements, including some of my own research.

ALS should absolutely be considered as a legitimate medical condition to use cannabis for medical purposes.

Thank you for your time and attention.

Sincerely,



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