RESEARCH ARTICLE

Safety and efficacy of dimethyl fumarate in ALS: randomised controlled study

Steve Vucic¹, Robert D. Henderson², Susan Mathers³, Merrilee Needham⁴,⁵,⁶, David Schultz⁷, Matthew C. Kiernan⁸ & the TEALS study group*

¹Brain and Nerve Research Centre, Concord Clinical School, Concord Hospital, University of Sydney, Sydney, Australia
²Department of Neurology, Royal Brisbane & Women’s Hospital, Brisbane, Australia
³Department of Neurology, Calvary Health Care Bethlehem, Caulfield South, Australia
⁴Department of Neurology, Fiona Stanley Hospital, Murdoch University, Murdoch, Australia
⁵Perron Institute for Neurological and Translational Science, Nedlands, Australia
⁶University of Notre Dame, Fremantle, Australia
⁷Flinders Medical Centre, Bedford Park, Australia
⁸Brain and Mind Centre, University of Sydney, Sydney, Australia

Correspondence
Steve Vucic, Westmead Clinical School, University of Sydney, Corner Darcy and Hawkesbury Road, Westmead, NSW 2045, Australia. Tel: +612 97678447; Fax: +61 02 97 678479; E-mail: steve.vucic@sydney.edu.au

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10Study group members are listed above the references.

Abstract

Objective: Neuroinflammation is an important pathogenic mechanism in amyotrophic lateral sclerosis (ALS), with regulatory T cells (Tregs) mediating a slower rate of disease progression. Dimethyl fumarate enhances Treg levels and suppresses pro-inflammatory T cells. The present study assessed the safety and efficacy of dimethyl fumarate in ALS. Methods: Phase-2, double-blind, placebo-controlled randomised clinical trial recruited participants from May 1, 2018 to September 25, 2019, across six Australian sites. Participants were randomised (2:1 ratio) to dimethyl fumarate (480 mg/day) or matching placebo, completing visits at screening, baseline, weeks 12, 24 and 36. The primary efficacy endpoint was a change in Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) at week 36. Secondary outcome measures included survival, neurophysiological index (NI), respiratory function, urinary neurotrophin-receptor p75 and quality of life.

Results: A total of 107 participants were randomised to dimethyl fumarate (n = 72) or placebo (n = 35). ALSFRS-R score was not significantly different at week 36 (C0.38 [C0.0.03 to 1.52, p = 0.41]). Dimethyl fumarate was associated with a reduced NI decline week 36 (differences in the least-squares mean: 0.84 [C0.0.51 to 2.22, p = 0.22]). There were no significant differences in other secondary outcome measures. Safety profiles were comparable between groups. Interpretation: Dimethyl fumarate, in combination with riluzole, was safe and well-tolerated in ALS. There was no significant improvement in the primary endpoint. The trial provides class I evidence for safety and lack of efficacy of dimethyl fumarate in ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder of the human motor system, with a mean survival of 3–5 years. At a pathophysiological level, ALS is a multistep process, mediated through complex interactions of genetic, epigenetic, and environmental factors. At present, riluzole (an anti-glutamatergic agent) and edaravone (a free radical scavenger that reduces oxidative stress) are clinically available for the treatment of ALS, and both exert modest benefits. More recently, masitinib (a microglial agent) and sodium phenylbutyrate (PB) and taouroursodeoxycholic acid (TUDCA) [AMX 035] have also shown modest effects in patients with rapidly progressive ALS, although these agents are not clinically available.

Neuroinflammation has emerged as a potential mechanism contributing to ALS pathophysiology, with infiltrating T lymphocytes leading to immune dysregulation and neuronal degeneration. Studies using transgenic mutant SOD1 mice (mSOD1) identified the presence of anti-inflammatory cytokines in the early stages of ALS, mediated
through central nervous system microglia and astrocytes, whereas an increase in pro-inflammatory mediators was observed in the rapid phase of disease progression.\textsuperscript{13–16}

The early stages of ALS are marked by an upregulation of regulatory T cells (Tregs) and increased levels of the neuroprotective type 2 microglia (M2) phenotype.\textsuperscript{12,16} Together, Tregs and M2 macrophages increase the levels of anti-inflammatory cytokines, such as interleukin (IL)-4, IL-10 and transforming growth factor $\beta$; suppress the pro-inflammatory helper-T (Th1) cells and Th17 lymphocytes; and suppress the activation/expansion of CD4$^+$CD25$^+$ effector T lymphocytes.\textsuperscript{17}

Disease progression is associated with conversion to a neurotoxic inflammatory response dominated by type 1 microglia (M1) and dysfunctional Tregs, which can no longer suppress Th1 and Th17 lymphocytes, as well as increased levels of pro-inflammatory cytokines (IL-1$\beta$, IL-6, interferon [IFN]-$\gamma$ and tumour necrosis factor $\alpha$).\textsuperscript{11,12} A correlation between Tregs and the rate of disease progression has been previously established in ALS and was associated with neuroprotective and neurodegenerative effects in ALS mouse models and some clinical trials,\textsuperscript{16,18} although other clinical trials have not reported a clinic benefit despite an increase in percentage of Tregs.\textsuperscript{19}

Importantly, the loss of Tregs’ suppressive ability in patients with ALS was reported to be transient, and the ability was regained when they were expanded in a different environment.\textsuperscript{17,20} This was corroborated by studies demonstrating that passive infusions of expanded Tregs had a suppressive function and slowed disease progression in patients with sporadic ALS.\textsuperscript{21} Collectively, these results highlight Tregs as a potential novel therapeutic target for the treatment of ALS.

Dimethyl fumarate, an FDA-approved medication used for the treatment of relapsing-remitting multiple sclerosis,\textsuperscript{22,23} has been shown to alter immune response towards more anti-inflammatory subsets. Specifically, dimethyl fumarate increased the number of anti-inflammatory CCR3$^+$Th2 and Treg cells, shifting the pro- to anti-inflammatory ratio (Th2/Th1Th17 and Treg/Th1Th17), with a simultaneous reduction in pro-inflammatory T cells (CD4$^+$IFN-$\gamma$; CD8$^+$IFN-$\gamma$), thus highlighting its potential as a neuroprotective agent.\textsuperscript{17–19} As such, the objective of this phase 2, randomised, placebo-controlled, multicentric, double-blind study was to assess the efficacy and safety of dimethyl fumarate in patients with ALS.

**Methods**

**Study design and participants**

This phase 2 study was conducted at six academic medical centres, coordinated through ALS Trials Australia. The study duration was 42 weeks: 14 days of screening; a 36-week treatment period; and 4-week follow-up period. The study was approved by the Human Research Ethics Committee and Research Governance Office at the lead site (Westmead Hospital; ethics number: HREC/17/WMEAD/353). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Participants provided written informed consent before the study began. The study protocol was published.\textsuperscript{24}

Men and women aged 18–85 years (inclusive) with a diagnosis of possible, probable, or definite sporadic ALS, per the Awaji criteria,\textsuperscript{25} and using standard clinical assessment criteria were enrolled in the study. Patients were deemed eligible if their disease duration was <24 months at the time of enrolment, they had had magnetic resonance imaging scans of the brain or spinal cord taken within 2 years prior to screening to exclude alternative disease processes, and they had a forced vital capacity (FVC) of $\geq$60% of predicted value adjusted for sex, age, and height. Eligible patients were allowed to use riluzole if they had been on a stable dose for $\geq$30 days prior to the screening visit.

The key exclusion criteria were: mechanical ventilation dependency; participation in other clinical trials; exposure to an investigational drug (within 12 weeks prior to screening); taking immunosuppressive medications; presence of a feeding tube at screening; familial history of ALS as reported by participant (separate genetic testing was not undertaken); active infectious disease (including hepatitis B or C, tuberculosis, or human immunodeficiency virus); unstable psychiatric or medical conditions; and clinically significant abnormal safety laboratory values. Women of childbearing potential were required to use medically acceptable contraception during the study. Pregnant or lactating women were excluded from the study.

**Randomisation and masking**

At screening, eligible patients were randomised 2:1 to receive either dimethyl fumarate or placebo. A computer-aided interactive web response system with random number generation was used, and this provided the patient medication kit/bottle number(s) of the blinded investigational product to be dispensed. All randomisation procedures were managed by a third-party vendor (Cenduit, India). The investigational product and placebo tablets were identical in physical appearance (matched in size, colour, presentation, and taste). All the patients and personnel at all the participating sites were blinded to the treatment assignments. Dimethyl fumarate was provided to the participating sites by Biogen Idec Australia via a...
central supplier and dispensed by the clinical study pharmacist or equivalent at each site.

**Procedures**

Dimethyl fumarate was administered at a dose of 240 mg twice daily through to week 36. Patients in the placebo group received matching capsules orally twice daily.

Eligible patients underwent a 14-day screening period. All the baseline characteristics were assessed at week 0. The neurological and physical examinations were performed at Weeks 12, 24, 36, and 40. Scores for the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R),\(^\text{26}\) the neurophysiological index (NI), the split-hand index (SI), the FVC, the sniff nasal inspiratory pressure (SNIP), and the Medical Research Council (MRC), along with urine neurotrophin-receptor p75 levels, were assessed at all timepoints. The revised ALS-specific QoL (ALSSQoL-R) score was assessed at Weeks 12, 36, and 40. Survival status was assessed at week 36.

Haematological and biochemical laboratory evaluations and adverse events were recorded throughout the study duration. An independent data safety management board reviewed the safety aspects and monitored the outcome of the study (Chair Professor David Brown, Professor Domini Dwyer and Professor Golo Ahlenstiel).

**Outcomes**

The primary endpoint was the change in the ALSFRS-R total score at 36 weeks. The ALSFRS-R score assesses the rate of disease progression based on four major functional domains: bulbar function (speech, swallowing, salivation); fine motor tasks (cutting, handling utensils, dressing and hygiene); gross motor tasks (walking and climbing stairs) and respiratory function (dyspnoea and orthopnoea).

Secondary endpoints included survival (in months), defined as the time from symptom onset to death or tracheostomy, lower motor neuron dysfunction (as assessed by MRC, NI and SI scores); respiratory dysfunction (FVC (%)) and SNIP); urinary levels of neurotrophin-receptor p75 (biomarker for ALS)\(^\text{27}\) and quality of life (ALSSQoL-R score). While in the original protocol primary and secondary endpoints were to be analysed at week 40, given that treatment ended at week 36, all efficacy endpoints were re-analysed at week 36. The protocol amendment was finalised after statistical planning, but prior to breaking the blind. This was documented and approved by the relevant ethics committee(s). Safety assessments included, adverse events, serious adverse events, clinical laboratory values (haematology, biochemistry and urinalysis), vital sign measurements and physical examination. ALS disease progression was not considered to be an adverse event.

**Statistical analysis**

Based on previous clinical experience, it was assumed that the mean difference in the ALSFRS-R between active and control groups was 7 (with SD of 8%) at 36 weeks. A clinically important response rate on Tecfidera was defined as 0.9. Given a 2:1 (dimethyl fumarate: placebo) allocation, 90% power and 5% statistical significance rate, it was estimated that a total of 72 (dimethyl fumarate, 48: placebo, 24) evaluable participants would need to be recruited for the study.

Statistical analyses were performed using SAS software version 9.4 (Cary, NC, USA). For continuous variables, results were presented as the number of contributing observations (n), mean, SD, median, minimum, and maximum. The number and percentage for each category were presented in the summary of categorical variables. The change from baseline at week 36 was calculated for all parameters (except survival) and the between-treatment difference was analysed using one-sided p-values at 5% significance level and 95% confidence intervals. Survival analysis was performed using a Kaplan–Meier curve. Multiple imputation (MI) method was used for missing data. A statistically significant result was regarded as a p < 0.05. This study is registered with Australia and New Zealand Clinical Trial Registry, number ACTRN12618000534280 (Date submitted 5/04/2018, 1st patient recruited 1 May 2018).

**Results**

Between 1 May 2018 and 25 September 2019, 117 participants were screened and 109 were enrolled on the TEALS study. Of these, 107 participants were randomised to receive either dimethyl fumarate or placebo in a ratio of 2:1 ratio. In total, 70% of participants (n = 75) completed the study, with discontinuation rates being similar for dimethyl fumarate (N = 50, 30.6%) and placebo (N = 25, 28.6%) cohorts (Fig. 1). Although 56% of drop-out patients occurred between weeks 12–24 (59% tecfidera; 41% placebo), and 44% between weeks 24–36 (60% tecfidera; 40% placebo), the study was adequately powered to detect the prespecified difference in the primary endpoint between dimethyl fumarate and placebo. The median treatment duration was 24.1 weeks, with the majority of participants being male (65.4%) and Caucasian (88.8%). The mean age of participants at recruitment was 60±10.2 years. The demographic and baseline characteristics were comparable between the dimethyl fumarate and placebo groups (Table 1).
Efficacy

Primary endpoint

The primary endpoint was the change in ALSFRS-R score at week 36. As explained in the methods, in the original protocol primary and secondary endpoints were to be analysed at week 40, however, given that treatment ended at week 36, all efficacy endpoints were analysed at week 36. There were no significant difference in the ALSFRS-R score between the dimethyl fumarate and placebo groups at week 36. Specifically, the between-group least-squares mean difference (ΔLSM) was −1.12 (−3.75 to 1.52, \( p = 0.41 \), Table 2, Fig. 2) at week 36. Data imputation (using the MI method) was undertaken in 24 participants (22.4%), 16 in the dimethyl fumarate (22.2%) group and 8 (22.9%) in placebo group. The ALSFRS-R was imputed from week 12 in 13.1% of participants (9.7% dimethyl fumarate, 20% placebo) and from week 24 in 9.3% of participants (12.5% dimethyl fumarate, 3% placebo).
Table 1. Demographic and baseline characteristics (intention-to-treat population1).

|                      | Placebo (n = 35) | Dimethyl fumarate (n = 72) |
|----------------------|------------------|-----------------------------|
| **Age, mean (SD), years** | 58.7 (11.0) | 60.1 (9.8) |
| **Sex n (%)**         |                  |                             |
| Male                  | 23 (66)          | 47 (65)                     |
| Female                | 12 (34)          | 25 (35)                     |
| **Race, n (%)**       |                  |                             |
| White                 | 31 (89)          | 64 (89)                     |
| Asian                 | 2 (6)            | 3 (4)                       |
| Other                 | 2 (6)            | 4 (6)                       |
| **Smoking status**    |                  |                             |
| Smokers, n (%)        | 4 (11)           | 9 (13)                      |
| Number of cigarettes per day, mean (SD) | 16.3 (9.5) | 9.0 (5.7) |
| **Diagnosis duration, mean (SD), days** | 201.6 (165.5) | 242.5 (194.9) |
| **Disease onset type, n (%)** |          |                             |
| Bulbar                | 4 (11)           | 13 (18)                     |
| Limb                  | 30 (86)          | 59 (82)                     |
| Both                  | 1 (3)            | 0                           |
| **ALS diagnosis, n (%)** |                  |                             |
| Definite              | 12 (34)          | 17 (24)                     |
| Probable              | 15 (43)          | 39 (54)                     |
| Possible              | 8 (22)           | 16 (22)                     |
| **ALSFRS-R total score, mean (SD)** | 38.7 (4.5) | 38.6 (5.5) |
| **Rate of disease progression/month** | 2.2 (2.3) | 2.1 (1.8) |
| **NI, mean (SD)**     | 2.6 (4.5)        | 2.5 (5.4)                   |
| **MRC score, mean (SD)** | 4.0 (4.2) | 3.9 (3.7) |
| **%FVC, mean (SD)**   | 85.2 (15.5)      | 90.3 (16.5)                 |
| **ALSSQoL score, mean (SD)** | 5.7 (0.7) | 5.3 (0.9) |
| **Use of riluzole, n (%)** | 27 (77) | 58 (81) |

ALS, amyotrophic lateral sclerosis; ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; ALSSQoL, ALS-specific quality of life; FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; MRC, Medical Research Council; SI, split-hand index.

1The intention-to-treat population included all randomised patients.

Secondary endpoints

Dimethyl fumarate exerted a significant effect on the neurophysiological index score, a robust neurophysiological biomarker of disease progression in ALS28,29. The rate of decline in NI was reduced in the dimethyl fumarate group, with the ΔLSM 0.84 (−0.51 to 2.22, p = 0.22; Table 2), although this difference was not significant.

Table 2. Least squares mean change from baseline in primary and secondary endpoints at week 36 (last observation carried forward; intention-to-treat population)1.

|                      | Placebo | Dimethyl fumarate |
|----------------------|---------|-------------------|
| **ALSFRS-R score**   | -4.89 (0.80) | -6.0 (0.57) |
| **NPI**              | -1.25 (0.54) | -0.40 (0.45) |
| **SI**               | -0.13 (0.57) | -1.24 (0.41) |
| **%FVC**             | -2.27 (3.02) | 1.01 1.96) |
| **SNIP (cmH2O)**     | -8.07 4.94) | -6.85 3.16) |
| **Urinary neurotrophin-receptor p75 level (ng/ml creatinine)** | 0.30 0.85) | 1.35 (0.84) |

Data imputation was undertaken in 30 participants (28%), 18 in the dimethyl fumarate (25%) group and 12 (34%) in the placebo group. The NI was imputed from...
week 12 in 15.9% participants (13.9% dimethyl fumarate, 20% placebo) and from week 24 in 12.1% of participants (11.1% dimethyl fumarate, 14.3% placebo).

Additionally, there were no significant effects observed in other secondary endpoints, including SI, MRC scores, respiratory function, and quality of life scores (Table 2) at week 36. In addition, urinary neurotrophin-receptor p75 levels were comparable between the dimethyl fumarate and placebo groups at week 36 ($p = 0.27$, Table 2). Dimethyl fumarate did not demonstrate any beneficial effect on survival compared with placebo ($p = 0.09$; hazard ratio $>99$), with the majority of participants in the dimethyl fumarate group (93.8%) and all in the placebo group being censored.

In total, 86.0% of participants experienced at least one adverse event during the study (dimethyl fumarate: 90.3%; placebo: 77.1%; $p = 0.08$, Table 3). Most adverse reactions were mild and included cutaneous flushes, urinary tract infection and upper respiratory tract infection in the study population (Table 3). Treatment related adverse events (TEAEs), namely cutaneous flushing, was significantly more common in the dimethyl fumarate group and is in keeping with expectations. Serious adverse events were reported in 18.7% of participants (19.4% in dimethyl fumarate group and 17.1% in placebo group, Table 3). The serious adverse events were not related to the study treatment. It should be stressed that there were no significant differences between treatment groups for the incidence of serious TEAEs, TEAEs leading to permanent or temporary study drug discontinuation, TEAEs leading to death and unexpected TEAEs.

Six participants (5.6%) discontinued the study because of adverse events, five in dimethyl fumarate and 1 in placebo group. The most common adverse events resulting in treatment were nausea (one patient in each group) and abdominal pain (one patient each group). One patient died during the study (in active group), and this was related to (disease progression and unrelated to study drug). Analyses of laboratory data, vital signs, and physical findings did not reveal clinically relevant changes in either group throughout the study duration.

**Discussion**

Dimethyl fumarate was proposed as a possible disease-modifying drug in ALS, acting via modulation of regulatory T cell function, which has previously been shown to be associated with a reduced rate of disease progression.\(^{17}\)

Although the co-administration of dimethyl fumarate with riluzole was found to be safe and well-tolerated in sporadic ALS there was no significant difference between dimethyl fumarate and placebo in the primary endpoint, namely ALSFRS-R score, at week 36. There was no significant difference between dimethyl fumarate and placebo in secondary endpoints, including NI, survival and respiratory function. Taken together, the present phase 2, randomised,
Serious adverse events, although this needs verification in a larger trial. Dimethyl fumarate is a repurposed oral immunomodulatory agent, used in the treatment of relapsing-remitting multiple sclerosis. It exerts anti-inflammatory and antioxidant effects by binding to the transcription factor nuclear factor erythroid-derived 2-like 2 (Nrf2). Specifically, dimethyl fumarate was shown to exhibit a relative increase in Tregs and suppress cytotoxic (Th1/Th17) cells as well. In addition, dimethyl fumarate switches the molecular and functional phenotype of activated microglia from the pro-inflammatory to anti-inflammatory type, exerting neuroprotective effects. The preclinical and clinical effects of dimethyl fumarate formed a scientific rationale for assessing the potential clinical effectiveness of dimethyl fumarate in our ALS cohort.

The primary endpoint (ALSFRS-R) was not significantly different between dimethyl fumarate and placebo groups, thereby arguing against the clinical effectiveness of dimethyl fumarate in sporadic ALS. Additionally, the rate of decline in NI was less in the treated cohort, although this difference was not significant when implementing the multiple imputation method. The NI is a simple, reproducible and robust biomarker of lower motor neuron dysfunction and disease progression in ALS, and consequently would have utility in Phase 2 trials. It should be stressed that the present trial did not prespecify power calculations according to changes in NI. Future phase 2 trials could pre-specify NI as an outcome biomarker to determine efficiency of compound at an early stage of their development.

It should also be highlighted that the rate of decline in the ALSFRS-R score over 36 weeks was unexpectedly small, with a 5.66-point decline in the dimethyl fumarate group and a 4.24-point decline in the placebo group. Importantly, the decline in the ALSFRS-R was smaller when compared with recent ALS clinical trials reporting significant differences in the primary endpoints. Specifically, the calculated projected decline in ALSFRS-R at week 36 in the edaravone trial (MCI-186) was 7.52 (active)/11.25 (placebo), in the masitinib trial, the decline was 6.93 (active)/9.47 (placebo), and in the CENTAUR trial (Sodium Phenylbutyrate-Taurursodiol), the calculated decline was 11.16 (active)/14.94 (placebo), all being higher than the current trial. The previous trials selected for the “fast-ALS progressors,” which the present study did not, could explain the absence of clinical significance and lower rates of ALSFRS-R decline in the current trial. Future clinical trials should assess dimethyl fumarate efficacy in “fast progressing” ALS patients.

**Limitations**

A potential limitation of the current trial relates to immunotyping of the ALS patients during treatment.
Specifically, T-cell profiling was not undertaken and consequently, the absence of clinical effectiveness could have been related to the failure of modulating effects of dimethyl fumarate on T cell populations in the current ALS cohort. Future studies should stratify patients based on T cell profiles to determine whether dimethyl fumarate could be more effective in participants exhibiting a greater imbalance between the cytotoxic and helper T cells. In addition, monitoring the biological effects of dimethyl fumarate on T cell populations may help identify a responding cohort of ALS patients. Separately, it could be argued that the higher-than-expected discontinuation rate influenced the results of the TEALS study. This seems unlikely as the power calculations indicated that 72 participants were required and a total of 107 participants were randomised of which 75 completed the study, thereby ensuring adequate study power.

Conclusion

In conclusion, the TEALS study established that dimethyl fumarate was safe and well-tolerated in sporadic ALS participants. There were no significant effects of dimethyl fumarate on primary and secondary endpoints. Consequently, the present trial provides class I evidence for safety and lack of efficacy of dimethyl fumarate in ALS.

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Author's Contribution

Dr SV- Study design, conducting experiments, patient recruitment, data analysis, writing of manuscript. Dr RH- Study design, conducting experiments, data analysis, critiquing of manuscript. Dr SM- Study design, conducting experiments, critiquing of manuscript. Dr MN- Study design, input in data analysis, critiquing of manuscript. DR DS- Study design, input in data analysis, critiquing of manuscript. Prof MCK- Study design, critiquing manuscript, patient recruitment input in data analysis.

Conflict of Interests

Prof SV- reports received honoraria from Merck Serono Australia, advisory board Biogen Idec Australia. Unrelated to this manuscript. Dr RH reports no disclosures. Dr SM reports no disclosures. Dr DS reports no disclosures. Prof MCK- reports no disclosures.

Teals Study Group

Linda Mekhail, Julie Ryder, Bronwen Orden, Parvathi Menon, Nathan Pavey, Mana Higashihara, Thanuja Dharmadasa, William Huynh, Eleanor Ramsay, Margaret Zoing, Sreepa Mazumder, Nicolette Thornton, Lesley Ann Hall, Peter Allcroft, Marie Toubia and Susan Hopkins.

References

1. Kiernan MC, Vucic S, Talbot K, et al. Improving clinical trial outcomes in amyotrophic lateral sclerosis. Nat Rev Neurol. 2021;17:104-118.
2. Kiernan MC, Vucic S, Cheah BC, et al. Amyotrophic lateral sclerosis. Lancet. 2011;377:942-955.
3. Vucic S, Westeneng HJ, Al-Chalabi A, Van Den Berg LH, Talman P, Kiernan MC. Amyotrophic lateral sclerosis as a multi-step process: an Australia population study. Amyotroph Lateral Scler Frontotemporal Degener. 2019;20:532-537.
4. Vucic S, Higashihara M, Sobue G, et al. ALS is a multistep process in South Korean, Japanese, and Australian patients. Neurology. 2020;94:e1657-e1663.
5. Eisen A, Braak H, Del Tredici K, Lemon R, Ludolph AC, Kiernan MC. Cortical influences drive amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2017;88:917-924.
6. Bensimon G, Lacomblez L, Meininguer V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. N Engl J Med. 1994;330:585-591.
7. Lacomblez L, Bensimon G, Leigh PN, Giullet P, Meininguer V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Lancet. 1996;347:1425-1431.
8. Writing Group EM-ASG. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2017;16:505-512.
9. Mora JS, Genge A, Chio A, et al. Masitinib as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a randomized clinical trial. Amyotroph Lateral Scler Frontotemporal Degener. 2020;21:5-14.
10. Paganoni S, Macklin EA, Hendrix S, et al. Trial of sodium phenylbutyrate-taurursodiol for amyotrophic lateral sclerosis. N Engl J Med. 2020;383:919-930.
11. Beers DR, Appel SH. Immune dysregulation in amyotrophic lateral sclerosis: mechanisms and emerging therapies. Lancet Neurol. 2019;18:211-220.
12. Thonhoff JR, Simpson EP, Appel SH. Neuroinflammatory mechanisms in amyotrophic lateral sclerosis pathogenesis. Curr Opin Neurol. 2018;31:635-639.
13. Beers DR, Zhao W, Liao B, et al. Neuroinflammation modulates distinct regional and temporal clinical responses in ALS mice. Brain Behav Immun. 2011;25:1025-1035.
14. Beers DR, Henkel JS, Zhao W, et al. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain. 2011;134:1293-1314.

15. Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc Natl Acad Sci USA. 2008;105:15558-15563.

16. Sheean RK, McKay FC, Cretney E, et al. Association of regulatory T-cell expansion with progression of amyotrophic lateral sclerosis: a study of humans and a transgenic mouse model. JAMA Neurol. 2018;75:681-689.

17. Beers DR, Zhao W, Wang J, et al. ALS patients’ regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity. JCI Insight. 2017;2:e89530.

18. Rajabinejad M, Ranjbar S, Afshar Hezarkhani L, Salari F, Gorgin Karaji A, Rezaianesh A. Regulatory T cells for amyotrophic lateral sclerosis/motor neuron disease: a clinical and preclinical systematic review. J Cell Physiol. 2020;235:5030-5040.

19. Camu W, Mickunas M, Veyrune J-L, et al. Repeated 5-day cycles of low dose aldesleukin in amyotrophic lateral sclerosis (IMODALS): a phase 2a randomised, double-blind, placebo-controlled trial. EBioMedicine. 2020;59:102844.

20. Liu J, Wang F. Role of neuroinflammation in amyotrophic lateral sclerosis: cellular mechanisms and therapeutic implications. Front Immunol. 2017;8:1005.

21. Thonhoff JR, Beers DR, Zhao W, et al. Expanded autologous regulatory T-lymphocyte infusions in ALS: a phase I, first-in-human study. Neurol Neuroimmunol Neuroinflamm. 2018;5:e465.

22. Gold R, Kappos L, Arnold DL, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med. 2012;367:1098-1107.

23. Fox RJ, Miller DH, Phillips JT, et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med. 2012;367:1087-1097.

24. Vucic S, Ryder J, Mehlak I, et al. Phase 2 randomized placebo controlled double blind study to assess the efficacy and safety of tecfidera in patients with amyotrophic lateral sclerosis (TEALS Study): Study protocol clinical trial (SPIRIT Compliant). Medicine. 2020;99:e18904.

25. de Carvalho M, Dengler R, Eisen A, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol. 2008;119:497-503.

26. Cedarbaum JM, Stambler N, Malta E, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999;169:13-21.

27. Shepheard SR, Wuu J, Cardoso M, et al. Urinary p75 (ECD): a prognostic, disease progression, and pharmacodynamic biomarker in ALS. Neurology. 2017;88:1137-1143.

28. de Carvalho M, Scotto M, Lopes A, Swash M. Quantitating progression in ALS. Neurology. 2005;64:1783-1785.

29. Huynh W, Dharmadasa T, Vucic S, Kiernan MC. Functional biomarkers for amyotrophic lateral sclerosis. Front Neurol. 2018;9:1141.

30. Yadav SK, Soin D, Ito K, Dhib-Jalbut S. Insight into the mechanism of action of dimethyl fumarate in multiple sclerosis. J Mol Med. 2019;97:463-472.

31. Jacobsen AB, Bostock H, Tankisi H. Following disease progression in motor neuron disorders with 3 motor unit number estimation methods. Muscle Nerve. 2019;59:82-87.