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**SYSTEMATIC REVIEW PROTOCOL**

# Protocol for a systematic review and meta-analysis of experimental models of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease with no cure and limited treatment options. There is therefore an urgent need for effective therapeutic interventions in this disease. This protocol outlines the strategy for a systematic review and meta-analysis to identify, from in vivo animal and human induced pluripotent stem cell (hiPSC) studies, potential therapeutic interventions for ALS. Our aim is to perform a comprehensive review of the ALS literature to compile a list of (1) candidate interventions and (2) target pathways that may be of therapeutic benefit in patients with ALS.

**KEYWORDS**

ALS, FTD, in vivo animal models, therapeutic intervention

## 1 | INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating disease with limited treatment options. The disease process is characterized by the selective degeneration of motor neurons leading to muscle denervation and subsequent atrophy. Patients progressively lose control over their bodies resulting in death, usually from respiratory arrest, usually within 3 to 5 years of a diagnosis. Current treatment strategies rely on supportive management and symptom control. Only one medication, riluzole, is licenced for clinical use. Riluzole is a sodium channel blocker and may have other effects through inhibition of N-Methyl-D-aspartic acid (NMDA) receptor signalling, glutamate release and uptake, which might serve to dampen neuroexcitation.<sup>1</sup> The effects of this drug are only modest, improving survival by 2 to 3 months and perhaps delaying the onset of ventilator dependence

following respiratory failure. There have been no further successful treatments since the identification of riluzole as a potential therapeutic intervention in ALS, there is therefore an urgent need for effective medications to treat this disease.

Systematic review and meta-analysis of ALS literature may help us to identify potential therapeutic interventions from animal model data with potential for clinical application. A recent systematic review focused on the efficacy of stem cell therapy in ALS<sup>2</sup> and previously, analysis of the literature from the SOD1-G93A transgenic mouse model of ALS<sup>3</sup> identified commonly investigated pathways in the pathophysiology of ALS. However, there has been no systematic review and meta-analysis, to our knowledge, examining the ALS literature as a whole, encompassing efficacy of all therapeutic interventions with the potential for clinical application. Furthermore, since the review of the SOD1-G93A mouse model of ALS, the field has

changed dramatically with a greater understanding of the genetic and pathological mechanisms underpinning the disease and the establishment of a variety of diverse animal models of the disease. We will therefore examine the in vivo ALS literature with a focus on identifying interventions and target pathways that may have a therapeutic benefit in ALS.

## 2 | APPROACH

A systematic review will be performed assessing interventions implemented in preclinical data from all in vivo models of ALS and frontotemporal dementia (FTD) (given the clinical and genetic overlap between the diseases) including (1) mammalian models (mouse and rat), (2) organisms with a central nervous system (*Drosophila*, *C. elegans* and Zebrafish) and (3) multicellular eukaryotic models such as yeast. The search strategy will also include data from (4) human, induced, pluripotent stem cells (iPSCs) taken from patients with ALS, due to their potential for a direct translational application of the results. Individual meta-analyses will then be performed for each of the interventions identified. Interventions will also be grouped by their target pathway for further subgroup analysis.

## 3 | OBJECTIVES

### 3.1 | PICOS framework

**Population:** in vivo studies in ALS and FTD.

**Intervention:** all therapeutic interventions.

**Comparison:** control or vehicle treatment group.

### 3.2 | Outcome measure

Primary outcome: mortality (spontaneous or euthanased); for hiPSCs, cell death.

Secondary outcomes: (1) behavioural (locomotor, circadian rhythm, memory, body weight), (2) biochemical (misfolded protein load, markers of cell stress) and (3) histological measures (integrity of motor neurons, axons, glia, astrocytes, neuromuscular junctions and muscle).

**Study design:** all study types where outcome in animals or cells exposed to the intervention is compared with that in animals or cells not exposed to the intervention.

## 4 | METHODS

Sources: databases: (1) PubMed, (2) Medline and (3) EMBASE

NB: there will be no publication date restrictions and no language restrictions.

Date of searches: April 8, 2016.

## 4.1 | Search method: Pubmed

### 4.1.1 | Search (1) animal model data

((“amyotrophic lateral sclerosis” or “motor neuron disease” or “frontotemporal dementia” or FTLD or FTD or MND or ALS) AND ((mouse or mice or murine) or rat or (drosophila or “fruit fly”) or “c. elegans” or “zebra fish” or yeast)) AND Animals[Mesh:noexp]

### 4.1.2 | Search (2) iPSC data

((“amyotrophic lateral sclerosis” or “motor neuron disease” or “frontotemporal dementia” or FTLD or FTD or MND or ALS) AND (iPSCs OR “stem cells”)) AND (( Animals[Mesh:noexp] OR Humans[Mesh] ))

## 4.2 | Search method: EMBASE

### 4.2.1 | Search (1) animal model data

((Amyotrophic lateral sclerosis or ALS or Motor neuron disease or MND or Frontotemporal dementia or FTD or FTLD) and (Mouse or murine or mice or rat or drosophila or fruit fly or c elegans or zebra fish or yeast)).af. and animal.sh.

### 4.2.2 | Search (2) iPSC data

((Amyotrophic lateral sclerosis or ALS or Motor neuron disease or MND or Frontotemporal dementia or FTD or FTLD) and (iPSCs or stem cells)).af. and human.sh.

## 4.3 | Search method: Medline

### 4.3.1 | Search (1) animal model data

((Amyotrophic lateral sclerosis or ALS or Motor neuron disease or MND or Frontotemporal dementia or FTD or FTLD) and (Mouse or murine or mice or rat or drosophila or fruit fly or c elegans or zebra fish or yeast)).af. and animal.hw.

### 4.3.2 | Search (2) iPSC data

((Amyotrophic lateral sclerosis or ALS or Motor neuron disease or MND or Frontotemporal dementia or FTD or FTLD) and (iPSCs or stem cells)).af.

## 4.4 | Screening

We will use the Systematic Review Facility online screening tool (app.syrf.org.uk). We will screen the title and abstract of each paper identified and for potentially relevant papers the full text will be retrieved, imported to EndNote and duplicate records will be discarded. Two independent reviewers will assess each paper (for screening and data extraction) with regards to the inclusion and exclusion criteria and a quality score and will extract data as described below. If reviewer concordance is <0.66 a third reviewer will assess the paper.

## 4.5 | Eligibility

### 4.5.1 | Inclusion criteria

- All therapeutic interventions where outcome is compared with that in a control or placebo group in ALS or FTD disease models.

- Types of model.
  - Genetic (knock out/in) OR drug induced (not combinations).
  - Yeast, Drosophila, Zebrafish, C. elegans, Mouse, Rat, human induced pluripotent stem cells (hiPSCs).

#### 4.5.2 | Exclusion criteria

- No control group.
- Clinical studies.
- Reviews.
- Letters and comments.
- Co-treatments.
- Combinations of genetic and pharmacological induction of phenotype.
- Cancer cell lines and all non-human iPSC lines.

#### 4.6 | Quality checklist

CAMARADES' study quality checklist, adapted as follows:

Nine items will be considered, and the median number of checklist items scored, and the interquartile range, will be calculated.

- Peer review publication.
- Statement of potential conflict of interests.
- Sample size calculation.
- Random allocation to group.
- Allocation concealment.
- Blinded assessment of outcome.
- Appropriate control group identified.
- Compliance with animal welfare regulations.
- Statement of temperature control.

Study characteristics to be extracted:

- Study ID: (1) author and (2) year
- Intervention: (1i) drug from list identified from clinical data (drop-down menu) or (2) other (free text box)
- Type of model: (1) which animal, (2) genetic or pharmacological induction, (3) which protein/mutation (3) gender (4) mated or non-mated or N/A, (5) familial or sporadic disease.
- Type of therapy: (1) immune, (2) genetic, (3) pharmacological, (4) environmental (e.g. diet/temperature), (5) cell.
- Target pathway: (1) Calcium homeostasis, (2) excitotoxicity, (3) protein turnover, (4) apoptosis, (5) regeneration (6) trophic factor signalling, (7) immunomodulation, (8) inflammation, (9) oxidative stress, (10) anion channel abnormalities, (11) lipid metabolism, (12) energy balance (including mitochondrial disruption and (13) axon transport.
- Mode of intervention delivery: (oral, intrathecal, intracerebroventricular, intraventricular, intraspinal, intraparenchymal dialysis catheter, intracranial cell transplantation/injection, subcutaneous, intravenous, intramuscular, intraperitoneal).
- Sample size.
- Duration of intervention (1) single or (2) multiple or (3) continuous.

- Natural death or euthanased or N/A.
- Outcome: (1) outcome measure (2) primary or secondary (3) value.

#### 4.7 | Statistical analysis

An individual meta-analysis will be carried out for each intervention identified and a subgroup analysis of interventions grouped by putative biological target will also be performed. Additional subgroup analyses will include (1) assessment of SOD1 G93A mouse model control-group survival data for evidence of genetic drift with time and (2) comparison of efficacy of treatments and targets separately in sporadic and familial models of disease.

The outcome measures will be plotted for each of the studies identified and included on a forest plot. Given the variability of model organisms included in the analysis, primary outcome data (survival summary data) will be calculated as described previously<sup>4</sup> and secondary outcome measures will be recorded in standardised mean differences (SMD), to allow meaningful comparisons between studies. SMD will be compared using Hedges g statistic, to account for bias from small sample sizes, using a random effects model. Survival summary measures and SMDs will be reported as odds ratios with 95% confidence intervals. Heterogeneity will be assessed for all outcome measures using  $I^2$  values, and a funnel plot and Egger's regression test will be used to assess publication bias. The summary data from each analysis will then be compared to the other meta-analyses on a separate forest plot and a hierarchy of candidate interventions will be identified.

#### Conflicts of interest

The authors declare the following conflicts of interest: M.M. is an editor for the journal: *Evidence Based Preclinical Medicine*.

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