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Interferon signalling as a potentional therapeutic target in amyotrophic lateral sclerosis and frontotemporal dementia – protocol for a systematic review and meta-analysis

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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 studies, potential therapeutic interventions for ALS cases in which an interferon response is induced. Our aim is to perform a comprehensive review of the ALS literature to compile a list of (i) interventions affecting interferon signalling pathways and (ii) specific interferon signalling pathway targets that may be manipulated for therapeutic benefit in patients with ALS. We will do this by assessing the pre-clinical literature for the effects of interferon signalling pathway manipulation on the following primary and secondary outcome measures comparing models of ALS/FTD to controls; the primary outcome measure being survival, and secondary outcome measures including histological, biochemical, and behavioural metrics. We will also carry out a structured quality assessment of the literature to provide recommendations for future studies.

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Interferon signalling as a potentional therapeutic target in amyotrophic lateral sclerosis and frontotemporal dementia – protocol for a systematic review and meta-analysis

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Date and location of protocol registration: April 2022, PROSPERO
Stage of review at time of registration: Search conducted

Abstract
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 studies, potential therapeutic interventions for ALS cases in which an interferon response is induced. Our aim is to perform a comprehensive review of the ALS literature to compile a list of (i) interventions affecting interferon signalling pathways and (ii) specific interferon signalling pathway targets that may be manipulated for therapeutic benefit in patients with ALS. We will do this by assessing the pre-clinical literature for the effects of interferon signalling pathway manipulation on the following primary and secondary outcome measures comparing models of ALS/FTD to controls; the primary outcome measure being survival, and secondary outcome measures including histological, biochemical, and behavioural metrics. We will also carry out a structured quality assessment of the literature to provide recommendations for future studies.

Keywords: ALS; MND; FTD; interferon/IFN signalling pathway; *in vivo* animal models; therapeutic intervention

Introduction:
The immune system plays a prominent role in the pathogenesis of many chronic diseases, including amyotrophic lateral sclerosis frontotemporal spectrum disorders (ALS-FTSD). These disorders exhibit, albeit heterogeneously, features of immune system dysfunction such as excessive inflammation, inefficient immune response, and autoimmunity, and recent studies have identified interferon signalling as a key player in this dysfunction.

Substantial clinical, genetic and pathological heterogeneity exists in ALS-FTSD including divergent mono-genetic drivers of disease, TDP-43 and non-TDP-43 related pathology, and temporal heterogeneity (e.g., increased inflammatory activation with end-stage disease). However, despite this heterogeneity, the underlying pathophysiology, at least in a substantial number of cases, appears to converge upon a shared mechanistic target of interferon signaling (Tam *et al*, 2019). Understanding these pathways in greater depth will provide us with a more targeted personalized medicine approach to ALS/FTD therapeutics, and improved stratification for clinical trials.

The impact of the interferon signaling pathway in the pathogenesis of ALS has recently been demonstrated in a pre-clinical study (Gerbino *et al*., 2020) reporting a survival benefit when mice harbouring an ALS-associated disease-causing mutation in *SOD1* are crossed with a *TBK1* mutant mouse that cannot mount an interferon (IFN) response. These double mutant (*SOD1/ TBK1)* mice have equivalent pathogenic protein misfolding to *SOD1* mutant mice, but lower levels of end stage inflammation that corresponded with a significantly extended lifespan. This finding directly implicates an inflammation-mediated deleterious effect of interferon signalling in the pathogenesis

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of ALS, and highlights potential therapeutic and diagnostic pathways that could be generated from further insights into ALS-related interferon signaling. For example, IFN inhibitors could be used to improve survival in patients with short disease duration and/or IFN signaling molecules could be used as biomarkers to identify patients with short disease duration to improve trial outcomes.

The role of the interferon signalling pathway in response to TDP-43-associated pathology demonstrated in another pre-clinical mouse study (Yu *et al.*, 2020) further illustrates the importance of the immune system in ALS. Here TDP-43, by triggering the release of mitochondrial DNA (mtDNA) into the cytoplasm, subsequently activates the cytoplasmic DNA-sensing cGAS/STING (cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING)) pathway. Furthermore, these authors show that the cGAS/STING pathway is directly responsible for neuroinflammation in response to aberrant TDP-43, as genetic deletion of STING mitigates disease in an ALS mouse model. Clinically, these findings suggest that the potential therapeutic application of inhibition of cGAS/STING could help mitigate inflammation-related neuropathology in ALS.

Here we outline a protocol for a systematic review, meta-analysis and structured quality assessment of the preclinical literature to compile a list of (i) interventions affecting interferon signalling pathways and (ii) specific interferon signalling pathway targets that may be manipulated for therapeutic benefit in patients with ALS-FTSD. We assess the literature for the effects of interferon signalling manipulation in studies comparing models of ALS-FTSD to controls, with the primary outcome measure being survival, and secondary outcome measures including histological, biochemical and behavioural metrics. We also carry out a structured quality assessment of the literature to provide recommendations for future studies. Taken together, these data better our understanding of the contribution of inflammation to ALS-FTSD pathogenesis, as well as highlight targets for further preclinical and clinical studies that aim to improve personalised therapies for people with ALS-FTSD.

Approach

A systematic review will be performed assessing all interventions targeting the interferon signalling pathway from preclinical *in vivo* models of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), given the clinical and genetic overlap between the diseases. The review will include (i) vertebrate models (mouse, rat and zebrafish) with inducible interferons, and (ii) invertebrate models whose genomes encode homologs of the interferon signalling pathway but from which no active interferons have been identified (*Drosophila, C. elegans*). Individual meta-analyses will then be performed for each of the interventions identified. Interventions will also be grouped by targeted pathways for further subgroup analysis.

Objectives

PICOS framework:

Population: *in vivo* preclinical studies modeling proteinopathies associated with ALS and FTD

Intervention: all therapeutic and/or genetic interventions targeting interferon signalling pathways

Comparison: control or vehicle treatment group

Outcome measure:

Primary outcome: mortality (spontaneous or euthanised)

Secondary outcomes: (i) behavioural (locomotor, circadian rhythm, memory); (ii) biochemical (misfolded protein load, markers of cell stress) and (iii) histological (cell count, protein accumulation, inflammatory infiltrate).

Study design: all study types in which outcome in animals exposed to the intervention is compared with that in animals not exposed to the intervention.

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Methods:
Sources: databases: 1. PubMed, 2. MEDLINE and EMBASE
There will be no publication date restrictions and no language restrictions.
Date of searches: 07/04/22

Search methods:

PubMed Central
((((((((((MND) OR "motor neuron* disease") OR ALS) OR "amyotrophic lateral sclerosis") OR FTD) OR "frontotemporal dementia")) AND (((((IFN) OR interferon) OR interferon-inducible) OR ISG) OR ISGs))) AND (((((((nematode OR worm OR "Caenorhabditis elegans" OR elegans)) OR (Drosophila OR "fruit fly" OR "fruit flies" OR "melanogaster")) OR (Mouse OR Mice)) OR Rat) OR (Fish OR Zebrafish OR "Danio rerio"))))) NOT Review

Ovid (MEDLINE and EMBASE) - 2599
((ALS or MND or FTD) and (IFN or interferon) and (elegans or drosophila or melanogaster or mouse or rat or fish or zebrafish or rerio)).af. not Review.pt.

3 resources were selected in Ovid:
(1) Journals@Ovid Full Text April 06, 2022.
(2) Embase 1974 to April 06, 2022.
(3) Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions 1946 to April 06, 2022.

Other sources
Obvious omission of papers that satisfy the inclusion criteria, but not identified by the search terms (e.g., studies referenced within included papers) can be included at the screening or data extraction phases, upon agreement of at least 2/3 screeners.

Screening and data extraction:
We will use the Systematic Review Facility online screening tool to conduct our review (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. Three independent reviewers (FMW/OMR/JMG) will assess whether each paper meets inclusion and exclusion criteria before data extraction. Papers will be included if at least 2/3 reviewers agree. The first reviewer (FMW) will perform data extraction on all screened papers and subsequent reviewers (JMG/OMR) will perform independent 10% checks at the data extraction stage, with discrepancies settled by discussion and consensus.

Eligibility:
Inclusion Criteria
- All therapeutic interventions targeting the interferon pathway and including an outcome that is compared with that in a control or placebo group in ALS or FTD disease models
- Types of model
  - Genetic (e.g., knock out/in) OR drug induced (not combinations)
  - Drosophila, Zebrafish, C. elegans, Mouse and Rat

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- Conference abstracts may be included if data can be extracted

Exclusion Criteria
- No control group
- Clinical and human studies
- Reviews
- Letters and comments
- Co-treatments (polypharmacy)
- Combinations of genetic and pharmacological induction of phenotype
- Cell culture and yeast studies

Quality checklist:

CAMARADES' study quality checklist, adapted as follows:
9 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: (i) author and (ii) year
- Intervention (targeting interferon response): (i) drug or (ii) genetic
- Type of model: (i) which species, (i) genetic or pharmacological induction of phenotype, (iii) which protein/mutation (iii) sex (iv) mated or non-mated or N/A.
- Whether the intervention was validated (e.g., through qPCR of gene expression, or through western blot or ELISA demonstrating protein levels) where inferred to perturb interferon response
- Type of interferon response involved: e.g., Type I, Type III etc.
- Specific genes (e.g., sensing, signalling, ISGs), pathways (e.g., cGas/STING) and interferons (e.g., IFNb) mentioned
- Mode of intervention delivery: (e.g., enteral, parenteral, intrathecal)
- Sample size
- Time of intervention: (i) pre-symptomatic (ii) symptom onset (iii) late disease (iv) not stated
- Natural death or euthanised or N/A
- Outcome: (i) outcome measure (ii) primary or secondary (iii) value

Statistical analysis

An individual meta-analysis will be carried out for each intervention identified and subgroup analyses of interventions grouped by putative biological target will also be performed. 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 (Vesterinen *et al.*, 2014) and secondary outcome measures will

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be recorded in standardised mean differences (SMD), to allow for meaningful comparisons between studies. SMD will be compared using Hedges g statistic, to account for bias from small sample sizes, using a mixed effects model. Survival summary measures will be reported as hazard ratios with 95% confidence intervals. Heterogeneity will be assessed for all outcome measures using I^2 values. Publication bias will be assessed using a funnel plot and quantified by Egger's linear regression. 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. Study quality data will be summarized in frequency distributions and recommendations will be made based on areas that require improvements. Methods, where possible, will adhere to recommendations set out in the Cochrane handbook for systematic reviews.

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Conflicts of interest
The authors declare no conflicts of interest.

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