Study protocol:

ReLiSyR-MND (Repurposing Living Systematic Review – Motor Neuron Disease): A systematic approach to identify neuroprotective interventions for motor neuron disease.

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Version 1.2 1 Feb 2021
Uploaded to OSF 1 Feb 2021

1. Purpose

We aim to develop a systematic framework to identify, evaluate and report evidence of putative drugs in motor neuron disease (MND) or amyotrophic lateral sclerosis (ALS) to guide prioritisation for evaluation in clinical trials. We adopted a systematic approach of evaluating drug candidates which we had previously used to guide drug selection for the Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial (MS-SMART) (1) a peer-reviewed MRC-EME £2.8M funded multi-arm phase IIb randomised controlled trial comparing the efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis. These principles of drug selection were published by Vesterinen *et al.* in 2015 (2). The search was expanded in 2014 to guide selection for the first two arms of the Motor Neuron Disease Systematic Multi-arm Adaptive Randomisation Trial (MND-SMART) (3). This approach, which adopts a structured, systematic method combined with independent expert(s) evaluation, was designed to identify candidate drugs for evaluation in clinical trials for people with neurodegenerative diseases, including MND / ALS, on account of the compelling evidence for shared dysregulated pathways and processes across neurodegenerative disorders. Critically, the structured evaluation takes into account not only biological plausibility and efficacy but also safety and quality of previous studies. This includes adopting benchmark practice such as Delphi and PICOS framework. We now proposed to undertake an up to date and ongoing systematic and structured evaluation of (i) clinical studies in motor neuron disease and other neurodegenerative diseases which may share common pivotal pathways, (ii) studies of animal in vivo models of MND / ALS and Frontotemporal dementia (FTD) where there are significant overlap in pathology with MND / ALS and (iii) publications describing in vitro MND/ALS studies. The results will be used to guide independent expert panel discussions and pathway analysis to identify further potential candidate targets, pathways and therapeutic agents.

2. Overall strategy:
[(2, 3, 4)].

For the clinical systematic review, we will perform a living search of PubMed using our search string detailed in section 3. The search will be performed through Systematic Review Platform (SyRF, app.syrf.org.uk) with automated weekly updates.

Two reviewers will screen title and abstract of clinical publications for inclusion according to the criteria listed in section 4. We will incorporate a machine learning algorithm to automate this process if performance of the classifier is satisfied (sensitivity > 95%, specificity > 80%).

From each publication, data of interventions and diseases studied will be extracted by two reviewers. We will develop machine learning / text mining algorithms (Regular Expressions deployed in R and taking as source material title and abstract) to automate this process thus creating a live workflow summarising the published clinical literature.

This will provide a list of interventions tested in human clinical trials in motor neuron disease (MND), Alzheimer's disease (AD), frontotemporal dementia (FTD), Huntington's disease (HD), Parkinson's disease (PD) and multiple sclerosis (MS). The other neurodegenerative diseases were included in our search as they may share common pivotal pathways.

A second algorithm is used to select interventions which have been tested in at least one clinical study in MND; or have been tested clinically in at least two of the other specified diseases.

To prioritise data extraction for interventions suitable for evaluation in clinical trials in the near future, our clinical trial investigators will review the interventions filtered, excluding interventions which meet any of the following criteria:

(i) previously considered unsuitable by expert panel due to lack of biological plausibility, interventions with unfavourable safety profiles in MND patients and interventions tested more than 3 times in MND population;
(ii) interventions available over-the-counter as these may affect trial integrity;
(iii) compounds which are not feasible for the next arms due to supply issues, such as compounds not listed in the current version of the British National Formulary;
(iv) interventions without oral preparations; and
(v) interventions that are deemed by investigators to be unsafe/inappropriate for clinical trial in the current setting.

The remaining interventions are longlisted for data extraction. Meta data and outcome data will be dual extracted with focuses on PIOOR (study population; intervention; outcome measured; outcome; and risks of bias) from literature that used potential interventions. Annotation questions

are listed in section 5. The data will be used to generate efficacy, safety, study size and quality scores for each intervention, from which we will generate an overall drug product score.

In vivo systematic review will be built up on previous systematic review (4), with a new live PubMed search on SyRF. Like the clinical review, we will screen publications and use machine learning to assist with citation screening and annotation of interventions studied. From this, we will generate a list of animal in vivo publications studying longlisted interventions. Meta data and outcomes (as listed in section 5) will be extracted by two reviewers for publications that include studies with the potential interventions. Meta-analysis (see section 6) will likely be used to calculate standardised mean difference (SMD) or normalised mean difference (NMD) for each intervention if there are enough studies with enough animals.

In vitro systematic review will be conducted in a similar approach to the in vivo systematic review, with the exception that the in vitro review will be a de novo review.

The intervention scores and results from clinical, in vivo and in vitro studies will be reported and visualised on our online web application platform.

3. Living Search terms:

3.1 Human clinical studies:

Pubmed:

((((((multiple sclerosis OR alzheimer's disease OR frontotemporal dementia OR FTD OR fronto-temporal dementia OR parkinson's disease OR parkinsons disease OR huntingtons disease OR huntington's disease OR Alzheimer's disease OR alzheimers disease) AND Clinical Trial[ptyp])) AND ("2013/12/01"[Date - Create] : "3000"[Date - Create]))) AND Clinical Trial[ptyp])

3.2 Animal in vivo studies:

Pubmed:

“("2016/04/06"[Date - Publication] : "3000"[Date - Publication]) AND (((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])”

3.3 In vitro studies

Pubmed:

(((“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. Inclusion and exclusion criteria

4.1 Human clinical studies

4.1.1 Inclusion criteria

- Publications reporting qualitative or quantitative data for safety or efficacy or both, for any intervention, tested in a clinical study (including case reports, case series, cohort studies, interventional trials) in patients with motor neuron disease / amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementia or multiple sclerosis.

4.1.2 Exclusion Criteria

- Protocols for clinical trials if publication does not contain any data as stated above.
- Review articles including systematic reviews
- Pharmacokinetic studies in healthy volunteers
- Preventative studies (i.e. studies on healthy volunteers or those with prodromal disease states)
- Publications where disease type is not clearly specified including studies on patients with "parkinsonism" which is not otherwise specified or specified as a different parkinsonian disorder (Dementia with Lewy Bodies, Progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy). Parkinson's disease with dementia is included and should be categorised as Parkinson's disease.
- Publication reporting studies involving patients with multiple different diseases where the primary outcome is not provided for individual disease types.
- Publications describing studies reporting use of multiple treatments within a cohort without results for individual treatments.

4.2 Animal in vivo studies:

4.2.1 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 and Rat.

4.2.2 Exclusion criteria

- No control group.
- Clinical studies.
- Reviews.
- Letters and comments.
- Co-treatments.
- Combinations of genetic and pharmacological induction of phenotype.

4.3 In vitro studies

4.3.1 Inclusion criteria

- Publications reporting quantitative data on therapeutic interventions where outcome is compared to control group in in vitro and ex-vivo studies of MND/ALS models.

4.3.2 Exclusion criteria

- No control group.
- Clinical studies.
- Reviews.
- Letters and comments.
- Co-treatments.
- Combinations of genetic and pharmacological induction of phenotype.

5. Annotation

5.1 Human clinical study:

For each publication we extracted the following data:

5.1.1 Study details:

- Type of study: (1) Observational: case report; case series; cohort; cross sectional; other/not specified; (2) Interventional
- If intervention trial:
    - Clinical Trial Phase
- Clinical trial registration number(s)

5.1.2 Disease:

- Type of disease: (1) MND/ALS, (2) AD, (3) FTD, (4) HD, (5) MS (if MS, what type of MS – PPMS/SPMS/CIS/RRMS/progressive MS (other/not otherwise specified)/not specified) or (6) PD

5.1.3 Participants/Patients:

- Number of patients

5.1.4 Intervention:

- Intervention
- Dose
- Route
- Duration of treatment and follow up
- If intervention was given (i) for symptomatic management only or (ii) control of spasticity only?

5.1.5 Safety data:

For each intervention described in a publication, a safety score [S] is assigned:

- “not described”: 1 point,
- “SUSARs (Suspected Unexpected Serious Adverse Reactions) or mortality observed”: 1 point,
- “SAEs (Serious Adverse Events) only”: 2 points,
- “AEs (Adverse events) only”: 3 points,
- “No adverse effects reported”: 4 points.

Interventions are scored based on an excess of safety events compared with control group when available or with natural history when control group is not available.

5.1.6 Efficacy:

An efficacy score [E] is assigned based on primary outcome measure, and where this is not identified, score is assigned on mean efficacy score of all efficacy outcome measures reported:

- "not presented": 1 point,
- "definite (i.e. statistically significant) worsening": 1 point,
- "neutral": 2 points,
- "non-significant improvement": 3 points,
- "significant improvement": 4 points.

5.1.7 Study quality

Assessed against a combination of criteria developed from a Delphi process (5), the GRADE criteria (6) and the CAMARADES criteria (7) (Table 1) to give a potential maximum sum score of 24 points from which a final quality score of 1 to 4 is derived by dividing the sum quality score by 6).

Table 1 Quality checklist items and scoring: Each publication was scored against this checklist to produce a sum quality score. A quality score of 1-4 is derived by dividing the sum quality score by 6.

Quality checklist items	**Score 0**	**Score 0.25**	**Score 0.5**	**Score 0.75**	**Score 1**
Peer review publication	No				Yes
Statement of potential conflicts of interest	No				Yes
Sample size calculation	No				Yes
Random allocation to group	No				Yes
Allocation concealment	No				Yes
Blinded assessment of outcome	No				Yes
Outcome assessor blinded	No				Yes
Patient Blinded	No				Yes

Care provider blinded	No				Yes
Were the groups similar at baseline regarding the most important prognostic indicators?	No		Not clear		Yes
Were the eligibility criteria specified?	No		Not clear		Yes
Were point estimates and measures of variability presented for the primary outcome measures?	No		Not clear		Yes
Was there an intention to treat analysis?	No		Not clear		Yes
Incomplete accounting of patient and outcome events?	Yes		Not clear		No
Selective outcome reporting	Yes		Not clear		No
Other limitations	Yes		Not clear		No
Was selection of treatment and control groups drawn from the same population?	Definitely no; Not applicable	Probably no		Probably yes	Definitely yes
Can we be confident that patients received the allocation treatment?	Definitely no	Probably no		Probably yes	Definitely yes
Can we be confident that the outcome of interest was not present at start of the study?	Definitely no	Probably no		Probably yes	Definitely yes

Did the study stratify on variables associated with the outcome of interest or did the analysis take this into account?	Definitely no	Probably no		Probably yes	Definitely yes
Can we be confident in the assessment of the presence or absence of prognostic factors?	Definitely no	Probably no		Probably yes	Definitely yes
Can we be confident in the assessment of outcome?	Definitely no	Probably no		Probably yes	Definitely yes
Was the follow up of cohorts adequate?	Definitely no	Probably no		Probably yes	Definitely yes
Were co-interventions similar between groups?	Definitely no; Not applicable	Probably no		Probably yes	Definitely yes

Patient sample size: As an additional quality item, a study size [SS] score of 1-4 as stated below is assigned to each publication depending on the number of patients included in the study. For non-crossover studies, we use the sum of patients allocated the intervention of interest and control group (e.g. standard of care or placebo group). If multiple investigational medicinal products were tested, patients taking other active investigational medicinal products (i.e. not part of standard of care), are not taken into account for this scoring. For studies with crossover design, we use the total number of participants in the study.

Study size [SS] score:

- 1-10 patients: 1 point
- 11-100 patients: 2 points
- 101-1000 patients: 3 points
- 1001+ patients: 4 points

5.2 Animal in vivo model studies

Study characteristics to be extracted:

5.2.1 Intervention:

- Intervention name: (1) drug from list identified from clinical data (drop-down menu) or (2) other (free text box)
- Type of therapy: (1) immune, (2) genetic, (3) pharmacological, (4) environmental (e.g. diet/temperature), (5) cell.
- Dose of intervention
- Timing of intervention (1) pre-symptomatic or (2) post-symptomatic.

5.2.2 Cohort information:

- Type of model: (1) which animal, (2) genetic or pharmacological induction, (3) which protein/mutation.
- Sample size.

5.2.3 Outcome: (1) outcome measure (2) primary or secondary (3) value, measures of variability, time of measurement

5.2.4 Quality checklist

CAMARADES' study quality checklist, adapted as follows:

These 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.
- Selective outcome reporting
- Incomplete outcome data

5.3 In vitro studies

Study characteristics to be extracted:

5.3.1 Intervention:

- Intervention name: (1) drug from list identified from clinical data (drop-down menu) or (2) other (free text box)
- Type of therapy: (1) immune, (2) genetic, (3) pharmacological, (4) environmental (e.g. diet/temperature), (5) cell.
- Timing of intervention (1) pre-induction or (2) post-induction.

5.3.2 Model information:

- Type of model: (1) cell source (primary; stem cell derived; organoid), (2) what type(s) of cell, (3) co-culture
- Disease Induction: (1) genetic or pharmacological, (2) which protein/mutation
- Number of cell lines.

5.3.3 Outcome: (1) outcome measure (2) primary or secondary (3) value, measures of variability, time of measurement

5.3.4 Quality checklist

CAMARADES' study quality checklist, adapted as follows:

These 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 (calculation of number of cell lines).
- Random allocation to group.
- Allocation concealment.
- Blinded assessment of outcome.
- Appropriate control group identified.
- Compliance with animal welfare regulations.
- Statement of temperature control.
- Selective outcome reporting
- Incomplete outcome data
- Passage number reported

6. Meta-analysis:

6.1 Human clinical studies

For each drug listed in each publication, we will calculate a distance score (based on Euclidean distance) as follows:

$$distance\ score = \sqrt{(safety\ score)^2 + (efficacy\ score)^2 + (study\ size\ score)^2 + (quality\ score)^2}$$

We will calculate median distance score across publications for each drug.

We will then calculate a final drug score as follows:

$$Drug\ score = log_{10}(\text{number of publications} + 1) \times \text{median distance score}$$

We will then rank drugs according to these scores.

Separately, for each drug, we will calculate median subscores for efficacy, safety, study size and quality.

6.2 Animal in vivo studies and in vitro studies:

For animal in vivo studies and in vitro studies, we will generate forest plots summarising the treatment effect of each longlisted intervention based on study outcomes. In addition, we will summarise the effects of interventions where there are 3 or more publications in which that intervention has been tested reporting findings from at least 5 experiments. Depending on the nature of the outcomes reported we will use either standardised mean difference (SMD) or normalised mean difference (NMD) random effects meta-analysis with REML estimates of tau. Specifically, if fewer than 70% of outcomes are suitable for NMD analysis we will use SMD. Differences between groups of studies will be identified using meta-regression. We will perform additional subgroup analysis including and excluding SOD1 G93A mouse models for animal in vivo studies.

7. Workflow

The final version of this protocol will be deposited in the Open Science Framework and will initially be private to the investigators.

Systematic review projects for clinical, in vivo and in vitro researches will be created separately on SyRF, Publication data will be stored within SyRF Database. Using the title and abstract data from SyRF as source data, we will use automated text mining algorithms to annotate disease and intervention data with R. A summary of disease and drug will be generated and presented to clinical trial investigators on an online application using R shiny services. Using a second algorithm, we will filter interventions to select interventions studied in at least one clinical study in MND or studied clinically in two or more other disease. To prioritise data extraction for interventions suitable for evaluation in clinical trials in the near future, our clinical trial investigators will review the interventions filtered to produce a longlist, excluding drugs which meet any of the following criteria: (i) previously considered unsuitable by expert panel due to lack of biological plausibility, drugs with unfavourable safety profiles in MND patients and drugs tested more than 3 times in MND population; (ii) drugs available over-the-counter as these may affect trial integrity; (iii) compounds which are not feasible for the next arms due to supply issues, such as compounds not listed in the current version of the British National Formulary; (iv) drugs without oral preparations; and (v) drugs that are deemed by investigators to be unsafe/inappropriate for clinical trial in the current setting. We will generate a list of publications studying longlisted interventions. Reviewers will extract meta-data and outcome for these publications within SyRF. Similarly, we will

identify all the in vivo and in vitro publications studying longlisted interventions for data extraction. We will calculate scores for each intervention for clinical studies and perform analysis as listed in section 6 for in vivo and in vitro studies. We will report all the results of clinical, animal in vivo and in vitro review on an online application powered by R shiny services.

References:

1. Connick P, De Angelis F, Parker RA, Plantone D, Doshi A, John N, et al. Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial (MS-SMART): a multiarm phase IIb randomised, double-blind, placebo-controlled clinical trial comparing the efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis. BMJ open. 2018;8(8):e021944.
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3. Wong C, Macleod M. A systematic approach to drug repurposing in motor neuron disease. 2020 OSF. https://osf.io/nrh7x/?view_only=d25e9fa1a1934554af41e8483a19e6ec
4. Gregory JM, Waldron FM, Soane T, Fulton L, Leighton D, Chataway J, et al. Protocol for a systematic review and meta-analysis of experimental models of amyotrophic lateral sclerosis. Evidence-based Preclinical Medicine. 2016;3(2):e00023.
5. Verhagen AP, de Vet HC, de Bie RA, Kessels AG, Boers M, Bouter LM, et al. The Delphi list: a criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus. J Clin Epidemiol. 1998;51(12):1235-41.
6. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. BMJ. 2004;%19;328(7454):1490.
7. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke. 2004;35(5):1203-8.

Appendix 1: ReLiSyR-MND workflow

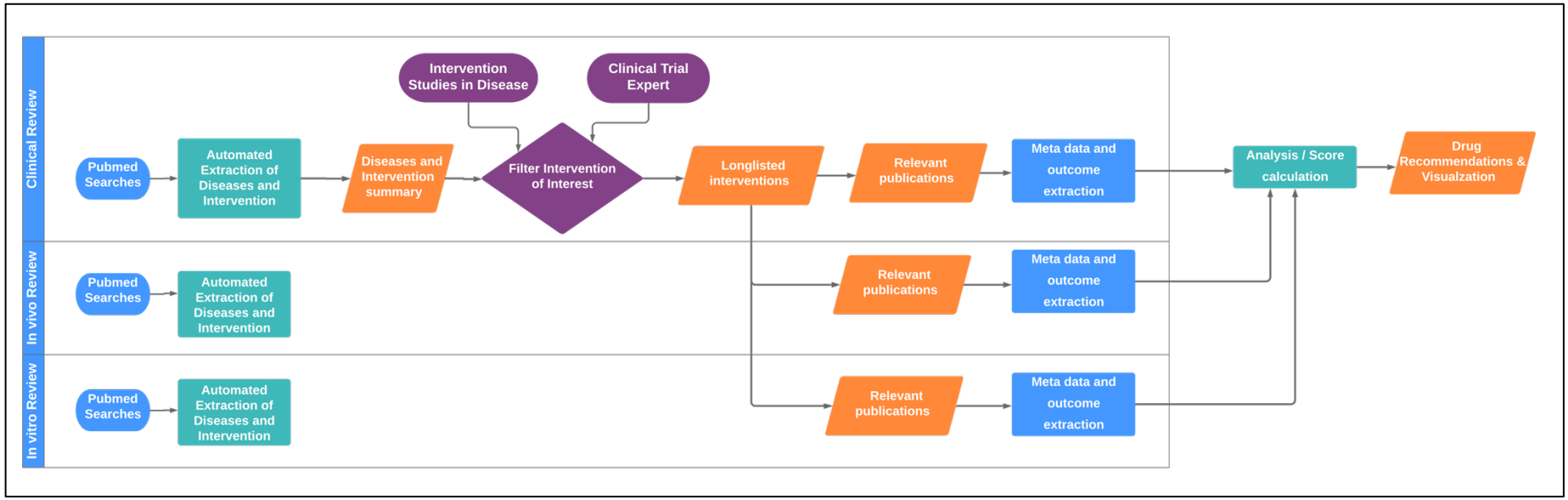

Figure 1 ReLiSyR-MND workflow. *ReLiSyR-MND consists of systematic review of (i) clinical literature of MND and other neurodegenerative diseases which may share similar pathways (Alzheimer's disease, Frontotemporal dementia (FTD), Huntington's disease, Multiple Sclerosis, Parkinson's disease), (ii) animal in vivo literature of MND and FTD models and (iii) in vitro literature of MND and FTD models.* ***Living Search****: We use the Systematic Review Facility (SyRF) platform , taking as its starting point automatic updating of the PubMed search.* ***Citation Screening****: Using a machine learning algorithm which has been trained and validated using human decisions, publications are screened for inclusion based on title and abstract.* ***Filtering drugs by inclusion logic****: Text mining approaches (Regular Expressions deployed in R and taking as source material title and abstract) are used to identify disease and drug studied. A second algorithm is used to identify drugs which have been tested in at least one clinical study in MND; or have been tested clinically in two of the other specified conditions.* ***Longlisting by trial investigators****: Trial investigators reviewed the drugs filtered, excluding drugs which met the following critera: (i) previously considered unsuitable by expert panel due to lack of biological plausibility, drugs with unfavourable safety profiles in MND patients and drugs tested more than 3 times in MND population; (ii) drugs available over-the-counter as these may affect trial integrity; (iii) compounds which are not feasible for the next arms due to supply issues, such as compopunds not listed in the current version of the British National Formulary; (iv) drugs without oral preparations; and (v) drugs that are deemed by investigators to be unsafe/inappropriate for clinical trial in the current setting.* ***Data extraction****: Our automation tool will generate a list of publications for longlisted interventions. Our team of reviewers extract data specified in our protocol on the SyRF platform from these publications. Each publication will be annotated by at least two reviewers, with any differences reconciled by a third reviewer.* ***Data Analysis****: Our automation tool analyses the meta data and outcome data extracted. For the clinical review, scores are calculated by our automation tool for each drug based on efficacy, safety, study size and quality of studies using our predefined metric. For preclinical reviews, an individual meta analysis will be carried out for each longlisted intervention.* ***Data visualisation and drug recommendations****: We will report current curated content arising from ReLiSyR-MND in our MND-SOLES-CT (Motor Neuron Disease - Systematic Online Living Evidence Summary for Clinical Trials; (https://camarades.shinyapps.io/MND-SOLES-CT/).*