SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	The Most Suitable Form to Delivery Antisense Oligonucleotides for Heritable Neurodegenerative and Neuromuscular Diseases Treatment: a Systematic Review	
2.	Authors (names, affiliations, contributions)	**Omar Paulino da Silva Filho**[1,2]**:** conception, design, acquisition, analysis and interpretation of data. **M. Leontien van der Bent**[2,3]**:** Conception, design, acquisition, analysis and interpretation of data. **Judith van Luijk**[4]**:** Conception, design, analysis and interpretation of data. **Derick G. Wansink**[3]: design, analysis and interpretation of data. **Roland Brock**[2]**:** Conception, design, analysis and interpretation of data. [1] CAPES Foundation, Ministry of Education of Brazil, Brasília – DF 70040-020, Brazil. [2] Department of Biochemistry, [3]Department of Cell Biology at Radboud Institute for Molecular Life Sciences, [4] SYRCLE at Central Animal Laboratory, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.	
3.	Other contributors (names, affiliations, contributions)	. **Alice Tillema** Medical Library, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.	
4.	Contact person + e-mail address	Omar Paulino da Silva Filho; omar.paulinodasilvafilho@radboudumc.nl Leontien van der Bent: Leontien.vanderbent@radboudumc.nl	
5.	Funding sources/sponsors	Science Without Borders (Omar Paulino) Prinses Beatrix Spierfonds (Leontien van der Bent)	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	April 2016, Nijmegen	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Title and abstract screening.	
	B. Objectives		
	Background		
10.	What is already known about this	Antisense oligonucleotide (AON) based therapy has	

	disease/model/intervention? Why is it important to do this review?	shown benefits in animal models for several of neurodegenerative and neuromuscular diseases (1), and also preliminary applicability in patients (2-4). The importance of this therapy is that the AONs modulate gene expression, in two different ways. Gapmer type AONs recruit RNaseH1 and mediate the degradation of the target RNA. The blocking type will sterically inhibit the binding of proteins, *e.g*. ribosomal subunits (1). Despite the promising results, oligonucleotides have poor residence time and stability in the blood circulation (1). It also has been identified that the size may compromise effective lipid membrane permeation (5). To overcome these physiological barriers and target specific cells, AONs formulated with delivery systems, *e.g.* cell-penetrating peptides (5). The delivery efficiency of AONs will depend on the formulation chemical structures, net charge, molar ratio and size (5, 6). Nonetheless, antisense therapy progresses slowly due to the lack of correlation regarding success in cell-based assays with that in animal models and also in patients (7). Testing the efficiency of delivered AONs in animal models result on an expensive and long-lasting experiments, and the humanized or mutated animal models may not express the ideal or desired phenotype (7-9). We aim with this systematic review to critically analyze the current studies involving the different delivery strategies of AONs in animal models for hereditable neuromuscular and neurodegenerative diseases to understand and efficiently plan our pre-clinical experiments. Consequently, we want to answer the following question: Which is the most appropriate delivery system for AONs in current animal models for neurodegenerative and neuromuscular disorders?	
	Research question		
11.	Specify the disease/health problem of interest	Animal models where neuromuscular or neurodegenerative disease is genetically induced.	
12.	Specify the population/species studied	All animal models for heritable neurodegenerative and neuromuscular diseases.	
13.	Specify the intervention/exposure	Delivery strategies of antisense oligonucleotides	
14.	Specify the control population	Naked oligonucleotides and/or mock treatment.	
15.	Specify the outcome measures	Primary outcomes: mRNA levels and exon skipping; protein expression; Possible secondary outcomes: body weight, motor function, performance in behavioral tests, biodistribution of AONs, survival, physiological muscle	

		and NMJ characteristics.	
16.	State your research question (based on items 11-15)	Which delivery system is the most effective for delivery of antisense oligonucleotides in animal models of heritable neurodegenerative and neuromuscular diseases?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of Science)	☒ Pubmed ☒ EMBASE ☒ Web of Science	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	MeSH/EMTREE terms were coupled with title/abstract/keyword terms to find all relevant articles. Disease, intervention and animal search strings are all combined with the Boolean operator AND. Thereby, all of these components will be present in the papers considered in our review.	
19.	Identify other sources for study identification	☒ Reference lists of included studies ☐ Books ☒ Reference lists of relevant reviews ☐ Conference proceedings, namely [type here] ☐ Contacting authors/ organisations, namely [type here] ☐ Other, namely [type here]	
20.	Define search strategy for these other sources	First, the identification will be based on the screening of the titles of related articles present on the reference lists of the included studies and relevant reviews. After the deduplication, the remaining relevant articles will be identified by their abstract.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full-text screening, both)	**First phase**: Screening by title and abstract. **Second phase**: screening by full-text of eligible articles.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	2 for both phases (Omar Paulino and Leontien van der Bent) + 1 extra in case of differences of opinion.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	**Inclusion criteria**: animal intervention studies. **Exclusion criteria**: clinical trials or non-intervention studies. Reviews out of topic or that do not present new data.	
24.	Type of animals/population (*e.g.* age, gender, disease model)	**Inclusion criteria**: all genetic animal models for heritable neurodegenerative and neuromuscular diseases: **Exclusion criteria**: Human study, *in vitro* study	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	**Inclusion criteria**: vectorized or non-vectorized antisense oligonucleotides. **Exclusion criteria**: co-interventions (for meta-analysis)	

26.	Outcome measures	**Inclusion criteria**: mRNA levels and/or exon skipping; protein expression. **Exclusion criteria:** none.	
27.	Language restrictions	**Inclusion criteria**: all languages **Exclusion criteria**: none	
28.	Publication date restrictions	**Inclusion criteria**: all publication dates **Exclusion criteria**: none	
29.	Other	**Inclusion criteria**: none **Exclusion criteria**: none	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase [title an abstract] 1. Not a primary study 2. Not an animal study 3. Not a correct animal model 4. Not an antisense treatment 5. Not a vectorized treatment 6. Selection phase Full text 1. Not the correct control group 2. Not the relevant outcome measure 3. Full-text article unretrievable 4.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	- Type of study - Duration of the study - Experimental groups - Number of animals in each group.	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	- Species - Gender - Age and body weight variation - Genetic background - Mutation or transgene	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	- Route of drug administration - Pharmacokinetics (distribution, absorption and clearance). - Pharmacodynamic (Ligand, receptor, dose, duration of action) - Antisense oligonucleotide modification - Formulation method Nanoparticle haracteristics - Delivery system - Net charge - Formulation method (N/P ratio, molecular ratio) - Particle size - Formulation stability	

35.	Outcome measures	Primary outcomes: mRNA levels and exon skipping; protein expression; Possible secondary outcomes: body weight, motor function, performance in behavioral tests, biodistribution	
36.	Other (*e.g.* drop-outs)	Was missing data handled appropriately? (were discrepancies reported)	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	Two reviewers (Omar Paulino and Leontien van der Bent). Discrepancies will be resolved by a third reviewer (to be specified).	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection, and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☒ By use of SYRCLE's Risk of Bias tool[4] □By use of SYRCLE's Risk of Bias tool, adapted as follows: □By use of CAMARADES' study quality checklist, e.g., 22 □By use of CAMARADES' study quality checklist, adapted as follows: □Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	mRNA levels – continuous data expressed as % of control exon skipping – continuous data expressed as % of control protein expression – continuous data expressed as % of control body weight (gain)– continuous data expressed in grams motor function (grip strength) – continuous data expressed in grams myofiber size – continuous data expressed in um^2 neuromuscular junction formation – continuous data expressed in % collapsed NMJ survival – continuous data expressed in %	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	From the included studies, the number of events or mean, standard deviation (SD) or standard error of mean(SE) as well as a total number of animals in each group. The data only presented in graphs will be measured using digital ruler software, wherever possible. The authors will be contacted and requested to provide the data in every case when quantified information extraction be impossible.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Two reviewers (Omar Paulino and Leontien van der Bent) will both extract data from half of the included studies. They will check the data extracted by the other Discrepancies will be resolved by a third	

		reviewer (to be specified).	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis will be performed (using Review Manager (version 5.3)) with subgroup analysis and sensitivity analysis for all outcome measures if possible. Otherwise descriptive summary.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Two reviewers will extract the data and an extra reviewer will be consulted in case of discrepancies (if any). A meta-analysis will be conducted by the reviewers whenever they identify more than two independent comparisons per outcome measure.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	All the outcome measures are continuous variables. They will express as mean difference (MD) or as standardized mean difference (SMD). Where outcomes are repeatedly measured at different points.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Sex, age at onset, administration route, delivery strategy, type of oligonucleotides, clinical severity at time of treatment (if reported)	
48.	Any sensitivity analyses you propose to perform	-	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple uses of control group)	-	
50.	The method for assessment of publication bias	We will visually inspect the Funnel plot to determine the publication bias if outcome contained, at least, ten or more studies.	

Final approval by (names, affiliations): Date:

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