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	Remyelination promoting therapies in multiple sclerosis animal models: a systematic review and meta-analysis	
2.	Authors (names, affiliations, contributions)	Benjamin Victor Ineichen[1, 2] Martin Hlavica[3] Marc Schneider[1] Nicolas Good[1] Andrin Good[1] Lisa Baumgartner[1] Gianluca Galeno[1] Carlijn Hooijmans[4] Rob DeVries[4] [1]University and ETH Zürich, Brain Research Institute, Switzerland [2]University Hospital Zurich, Department of Neurology, Switzerland [3]Cantonal Hospital St. Gallen, Department of Neurosurgery, Switzerland [4]SYRCLE at Central Animal Laboratory, Radboud University Medical Center, Nijmegen, the Netherlands	
3.	Other contributors (names, affiliations, contributions)	To be determined	
4.	Contact person + e-mail address	ineichen@protonmail.ch	
5.	Funding sources/sponsors	Swiss Multiple Sclerosis Society, Hartmann-Müller-Foundation, Desirée-and-Niels-Yde-Foundation, Swiss National Science Foundation	
6.	Conflicts of interest	The authors declare no conflict of interest	
7.	Date and location of protocol registration		
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Database search and abstract sorting completed, data extraction started	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Multiple sclerosis (MS) is a chronic neuro-inflammatory disease mainly starting in young ages. Whereas some immune system modulating therapies are available for the early disease stages in which immune cells infiltrate the central nervous system (CNS), no therapies exist for the progressive phase, defined by chronic demyelination and neurodegeneration. Therefore, finding therapies which promote myelin repair is top priority in neurological research. The four most commonly used animal models to	

		assess candidate drugs for their purely remyelinating properties are lysolecithin, ethidium bromide, cuprizone, and anti-galactocerebroside antibodies/complement. However, tracking overview about all assessed approaches in these models to enhance remyelination is very challenging. Hence, we aim at summarizing these potential interventions by this systematic review. Moreover, we are aiming at performing a meta-analysis on therapies which have been assessed more than once to estimate their efficacy. Finally, we plan to correlate these results with the outcome of clinical trials in human patients to determine parameters for successful clinical translation.	
	Research question		
11.	Specify the disease/health problem of interest	Multiple sclerosis	
12.	Specify the population/species studied	All species	
13.	Specify the intervention/exposure	Interventions which aim at improving remyelination	
14.	Specify the control population	No intervention or control intervention such as vehicle injection instead of drug injection	
15.	Specify the outcome measures	<u>Primary outcomes</u> •Remyelination outcomes such as electron microscopy/light microscopy analysis of remyelinated axons, optical density in myelin stainings, demyelinated area in myelin stains, etc. •Count of oligodendrocytes using stainings such as APC/CC1, Nogo-A, CA II, etc. •Count of oligodendrocyte precursor cells (OPCs) such as PDGFRα, NG2, Olig2/APC, etc. <u>Secondary outcomes</u> •Electrophysiological measures, behavioural performance, myelin protein analysis using western blots	
16.	State your research question (based on items 11-15)	1.) What is the current evidence for the efficacy of remyelinating interventions in the MS animal models lysolecithin, ethidium bromide, cuprizone, and anti-galactocerebroside antibodies/complement? 2.) Can we find a correlation between outcome in pre-clinical studies and outcome in clinical trials? 3.) Can we define parameters which can help to predict successful clinical translation?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	**X**MEDLINE via PubMed **X**Web of Science Core Collection **X**SCOPUS **X**EMBASE **X**Other, namely: go3R, BIOSIS	

18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	Consider supplementary search strings	
19.	Identify other sources for study identification	**X**Reference lists of included studies **X**Reference lists of relevant reviews	
20.	Define search strategy for these other sources	Examination of reference lists from relevant articles	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1) Pre-screening based on title and abstract 2) Full-text screening of the eligible articles, since a few thousand articles are available, full-text screening will be focused on abstract, method section and figures. In unclear cases, other parts will be considered as well.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	1.) 2 independent reviewers per abstract, abstracts/articles on which the reviewers disagree articles will be included in the full-text screening (over-inclusion approach) 2.) 2 independent observers per article. Differences will be solved through discussion or by consulting a third investigator.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Original works (including conference abstracts); use of an adequate control group (Vehicle only treatment) Exclusion criteria: Studies which did not investigate a therapy in these MS models will be excluded; a therapy is defined as a directly or indirectly and exogenous to the animal applied substance or intervention (e.g. studies which only investigate pathogenic aspects of MS or studies which only use transgenic approaches will be excluded). Reviews will be excluded but retained as a source for potential studies and for discussion.	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: all sexes, ages, rat and mice strains and one or more of the four types of models mentioned above Exclusion criteria: Studies where only transgenic animals were used, studies in which MS disease models are combined with other disease models (e.g. diabetic rats), in vitro approaches only (e.g. cerebellar rat slice cultures), mainly inflammatory MS animal models (e.g. EAE, TMEV)	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: all therapy regimens will be included (therapeutic, prophylactic, combined approaches) and therapies which aim at improving remyelination (histology/electron microscopy and/or myelinating or pre-myelinating cell counts (oligodendrocytes and OPCs))	

		Exclusion criteria: application (e.g. in case of proteins) via viral vectors (potential off target effects)	
26.	Outcome measures	Inclusion criteria: outcome measures related to remyelination or (pre-)myelinating cell counts	
27.	Language restrictions	Inclusion criteria: all languages	
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: screening of abstracts and full-text 1. Non-original article 2. No therapy tested 3. In vitro only 4. Only transgenic animals used 5. None of above mentioned animal models used Exclusion criteria for the meta-analysis: full-text screening 1. No data on remyelination 2. No reporting of quantitative data 3. Only G ratio as remyelination readout 4. No reporting of animal numbers or statistical variability (max. one e-mail will be sent to authors from studies in which no animal numbers and/or statistical variability are reported)	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year, title, journal, language	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of animals per group	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species, strain, sex, type of model	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Therapeutic/prophylactic/combined application regimen + molecule used, dose, administration route etc.	
35.	Outcome measures	All MS-related outcomes will be used. For remyelination outcomes, following extraction priority list is used: 1.) Electron microscopy: amount of remyelinated axons between treatment and control group(s) (disproportionally thinly myelinated axons) 2.) Toluidine blue/semithin section: amount of remyelinated axons between treatment and control group(s) (disproportionally thinly myelinated axons) 3.) Other stainings (e.g. MBP staining, sudan black staining): amount of remyelinated axons between treatment and control group(s) (disproportionally thinly myelinated axons) 4.) Other stainings (e.g. MBP, LFB, black gold, eriochrome, and others): lesion volume/area between treatment and control group(s)	

		5.) Magnetic resonance imaging (MRI): lesion volume/area 6.) Other stainings (e.g. MBP, LFB, black gold, eriochrome, and others): optical density within lesion between treatment and control group(s) For oligodendrocyte and OPC counts, stainings will be extracted with no priority	
36.	Other (*e.g.* drop-outs)		
	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	2	
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, adapted as follows: addition of one additional reporting item: is there reporting of randomization at any step?	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	For quantitative synthesis: continuous measurements will be extracted	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	First extraction from numbers in text or tables, second numbers from graphs using universal desktop ruler software (http://avpsoft.com/products/udruler/) by two independent reviewers. (If data could not be extracted from text or figures authors will be contacted via e-mail (max. 1 e-mail)).	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	2, by discussion, ultimately by a third reviewer	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	If possible, meta-analysis with subgroup analysis and sensitivity analysis for all outcome measures. Following meta-analyses will be performed: 1.) On the remyelination outcome (remyelination per se, measured by electron microscopy/histology). Since disproportionally thinly myelinated axons are the gold standard of measuring remyelination and therefore the most robust outcome measure, a second meta-analysis only including studies using this outcome readout will be performed (1.) to 3.) from the list from point 35). 2.) On oligodendrocyte cell counts. 3.) On OPC counts. In case numerical outcomes were quantified using different scales, mean and standard deviation will be calculated in the meta-analysis; additionally,	

		qualitative/descriptive analysis Exclusion criteria for quantitative synthesis: Papers only reporting qualitative data on remyelination, papers with only G ratio as quantitative remyelination readout due to the limited relative effect size (due to the differences in potential remyelination readout)	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	See 42.	
	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)	Standardized mean differences (SMD). If possible, we will do a sensitivity analysis in which we only include the studies for which we can calculate an NMD (normalized mean difference)	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model, Forest-plot for visualization	
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)	Subgroup analysis will only be performed in therapies which have been tested 4 or more times per outcome measure (remyelination, oligodendrocyte cell count or OPC count): •Species (rats vs. mice) •Sex •Prophylactic vs. therapeutic therapy regimen •MS animal model •Type of remyelination outcome/intervention	
48.	Any sensitivity analyses you propose to perform	To be determined	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	To be determined	
50.	The method for assessment of publication bias	Funnel plots or Eggers test in case of small study effects (n-based estimate of precision for your funnel plot)	
Final approval by (names, affiliations):		Benjamin Victor Ineichen University and ETH, Zürich \ Brain Research Institute Switzerland	Date: 24.02.2017