SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES
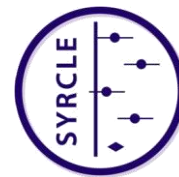
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Item #	Section/ item	Description	Check for approval
	General		
1.	Title of the review		
2.	Authors (names, affiliations, contributions)	Carlijn R Hooijmans [12]; designing and performing research, analysing data, writing paper Merel Ritskes-Hoitinga [1]; designing research, writing paper Gert-Jan Scheffer [2]; designing research, writing paper Florentine J Geessink [12]; performing research: data extraction, Quality assessment Departments of [1]SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) [2] Anesthesiology, [3] Medical Library, Radboud UMC Nijmegen, The Netherlands.	
3.	Other contributors (names, affiliations, contributions)	Alice Tillema [3], search strategy design Moira Bruintjes [12]; performing research; In- and exclusion Marleen Egberink [12]; performing research; in and exclusion and data extraction Sandra de Groot [2] ; performing research ; data extraction and quality assessment Marieke Schouten [2]; performing research ; data extraction and quality assessment	
4.	Contact person + e-mail address	Carlijn R Hooijmans; Carlijn.Hooijmans@radboudumc.nl	
5.	Date of protocol registration		
	Background		
6.	What is already known about this disease/ model/ intervention? Why is it important to do this review?	Analgesics are commonly used to manage pain in cancer patients. It has been suggested that there might be a relation between analgesics and the outgrowth of metastases. Opioids might increase, and NSAIDs decrease the risk of metastasis. Robust analysis of all preclinical evidence, however, has so far been lacking. Therefore, we will conduct a systematic review and meta-analysis on the effect of treatment with analgesics on metastasis in experimental animal models.	
	Objectives of this SR		
7.	Specify the disease / health problem of interest	Metastasis/ metastatic spread in experimental cancer	
8.	Specify the population /species studied	All species	
9.	Specify the intervention/exposure	Analgesic treatment (drugs used in the clinical setting)	
10.	Specify the control population	No analgesic treatment (placebo or sham or no intervention)	
11.	Specify the outcome measures	1) Number of metastasis 2) Metastasis incidence	
12.	State your research question (based	Does analgesic treatment reduce the number or incidence	

	on points 7-11)	of metastasis in experimental cancer	
Methods:			
	Search and study identification		
13.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	**X**MEDLINE via PubMed ☐Web of Science ☐SCOPUS **X**EMBASE ☐Other, namely: ☐Specific journal(s), namely:	
14.	Define electronic search strategies (*e.g.* use the step by step search guide [1] and animal search filters [2, 3])	When available, please add a supplementary file containing your search strategy: [*supplementary file 1 search analgesics*]	
15.	Identify other sources for study identification	**X**Reference lists of included studies ☐Books **X**Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
16.	Define search strategy for these other sources	Screening the reference lists for relevant titles and screening the abstracts of these relevant titles	
	Study selection phases		
17.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1) screening based on title and abstract 2) full-text screening of the eligible articles	
18.	Specify number of reviewers per screening phase	Each phase: 2 independent observers per article. Phase 1 : CH and MB screen all papers. Phase 2: CH and ME screen all papers.. Differences will be solved through discussion or by consulting a fourth investigator	
	Study selection criteria. Define all inclusion and exclusion criteria based on:		
19.	Type of study (design)	Inclusion criteria: Comparison of analgesic treatment versus no analgesic treatment on number of metastasis or metastasis incidence in animals with experimental cancer Exclusion criteria: Co-interventions/ contamination	
20.	Type of animals/ population (*e.g.* age, gender, disease model)	Inclusion criteria: animals with experimental cancer in which metastasis can develop Exclusion criteria: Co-morbidities, ex vivo, in vitro in silico, experimental cancer without metastasis	
21.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: analgesic treatment (also pre-treatment with analgesics of tumor cells before injection) Exclusion criteria: analgesics not used in the clinical setting	
22.	Outcome measures	Inclusion criteria: number of metastasis or metastasis incidence Exclusion criteria: weight of metastasis, surface covered with metastasis, number of occupied bones, number of invading cells	
23.	Language restrictions	Inclusion criteria: all languages Exclusion criteria: none	
24.	Publication date restrictions	Inclusion criteria: all publication dates	

		Exclusion criteria: none	
25.	Other	Inclusion criteria: Exclusion criteria: Reviews or non original papers	
26.	Sort and prioritize your exclusion criteria per selection phase	Selection phase 1: 1. Review 2. Human study 3. Not in vivo 4. No metastases/ only primary tumor 5. No control group 6. Combination therapy or contamination 7. Not about analgesics used in the clinic Selection phase 2: 1. Review 2. Human study 3. Not in vivo 4. No metastases/ only primary tumor 5. No control group 6. Combination therapy or contamination 7. Not about analgesics used in the clinic 8. No relevant outcome measure	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
27.	Study ID (*e.g.* authors, year)	Authors, title, year, language, contact author e-mail	
28.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of animals in experimental and control groups, presence of control group.	
29.	Animal model characteristics (*e.g.* species, gender, disease induction)	Animal species, strain, age or weight, gender, cancer model (transgenic or induced), type of cells/ drugs used to induce cancer, type of cancer, amount of cells, location of injection of tumor cells, type of anesthetics used to create model.	
30.	Intervention characteristics (*e.g.* intervention, timing, duration)	Type of analgesics, Route of administration, dose, frequency, timing relative to tumor cell injection, duration of treatment, type of control group	
31.	Outcome measures	Number of metastasis, incidence of metastasis	
32.	Other (*e.g.* drop-outs)	Age of sacrificing animals, anesthetics used for sacrificing, region of metastasis count	
	Risk of bias assessment (internal validity)		
33.	Specify the number of reviewers assessing the risk of bias in each study	2	
34.	Define criteria to assess the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias)	☐By use of SYRCLE's Risk of Bias tool [4] **X**By use of SYRCLE's Risk of Bias tool, adapted as follows: addition of 2 reporting items; 1) reporting of randomisation at any level 2) reporting of blinding at any level. ☐By use of CAMARADES' study quality checklist, e.g. [5]	

		□By use of CAMARADES' study quality checklist, adapted as follows: □Other, namely:	
	Collection of outcome data		
35.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/ dichotomous, unit of measurement)	Number of metastasis: continuous Incidence of metastasis: Continuous (% or number of animals in control and experimental group with metastasis)	
36.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	First extraction 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. 3 e-mails).	
	Data analysis/synthesis. Specify (per outcome measure):		
37.	How you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis with subgroup analysis and sensitivity analysis for all outcome measures	
38.	How the decision as to whether a meta-analysis will be performed will be made	A minimum of 4 articles per outcome measure is required No restrictions in terms of heterogeneity will be applied, instead, sources of heterogeneity will be investigated through sensitivity and subgroup analysis.	
	If a meta-analysis seems feasible/sensible, specify for each outcome measure:		
39.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Number of metastases: SMD Incidence of metastasis: RR	
40.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
41.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
42.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Type of drug (NSAID; opioid, local, paracetamol, ketamin) Species Sex (male, female, mixed) Region of metastasis Timing (before or after tumor injection) Duration of treatment	
43.	The method for assessment of publication bias	funnel plots, performing Duval and Tweedie's trim and fill analysis	
44.	Any sensitivity analyses you propose to perform	Weight of metastasis Tumor cells i.v versus local Pooling studies in which injected cancer cells are pre-treated with analgesics Pooling studies in which the median is recalculated to a mean	

		Transgenic animals versus induced cancer Study quality	
Final approval by (names, affiliations):		Carlijn Hooijmans	Date: 01-09-2014