SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/ item	Description	Check for approval
	General		
1.	Title of the review	Anti-tumor effects of Metformin in Animal Models of Hepatocellular Carcinoma: A systematic Review and Meta-Analysis	
2.	Authors (names, affiliations, contributions)	Juan Li[1]*, Pratika Y.Hernanda[2], Wichor Bramer[3], Maikel P. Peppelenbosch[1], Judith van Luijk[4], Qiuwei Pan[1]* [1]Department of Gastroenterology and Hepatology, Erasmus MC Cancer Institute, Erasmus University Medical Center and Postgraduate School Molecular Medicine, Rotterdam, The Netherlands; [2]Laboratory of Medical Genetics, Biomolecular Research Centre, Wijaya Kusuma University, Surabaya, Indonesia; [3]Medical Library, Erasmus MC, Rotterdam, The Netherlands; [4] SYRCLE at Central Animal Laboratory, Radboud University Medical Centre, Nijmegen, The Netherlands.	
3.	Other contributors (names, affiliations, contributions)	Dr. Rob BM de Vries SYRCLE at Central Animal Laboratory, Radboud University Medical Centre, Nijmegen, The Netherlands.	
4.	Contact person + e-mail address	Qiuwei Pan: Q.Pan@erasmusmc.nl; Juan Li j.li@erasmusmc.nl	
5.	Date of protocol registration	N.A.	
	Background		
6.	What is already known about this disease/ model/ intervention? Why is it important to do this review?	HCC is an aggressive malignant tumour of the liver and the third cause of cancer-related death worldwide. As Several preclinical observational studies have shown that metformin can decrease the risk of cancer in patients with diabetes. Therefore, there are accumulating studies about metformin's antitumor function in vitro and in vivo.	
	Objectives of this SR		
7.	Specify the disease / health problem of interest	Hepatocellular Carcinoma (HCC)	
8.	Specify the population /species studied	All animal species	
9.	Specify the intervention/exposure	Metformin intervention	
10.	Specify the control population	Placebo/no treatment	
11.	Specify the outcome measures	All tumour-related outcomes	
12.	State your research question (based on points 7-11)	Anti-tumour effect of Metformin on Hepatocellular carcinoma via animal models.	
	Methods:		

	Search and study identification		
13.	**Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)**	**☐MEDLINE via PubMed X Web of Science X SCOPUS X EMBASE X Other, namely: Medline (OvidSP), PubMed Publisher, Google Scholar ☐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: [insert file name]**	
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)**	**First phase screening by title and abstract, second phase screening by full-text**	
18.	**Specify number of reviewers per screening phase**	**2 for both phases +1 extra in case of differences of opinion**	
	Study selection criteria. Define all inclusion and exclusion criteria based on:		
19.	**Type of study (design)**	**Inclusion criteria: animal intervention studies (with control group). Studies will be included regardless of the methodological quality. Exclusion criteria: non-intervention studies, no control group**	
20.	**Type of animals/ population (*e.g.* age, gender, disease model)**	**Inclusion criteria: all animal models for hepatocellular carcinoma Exclusion criteria: humans**	
21.	**Type of intervention (*e.g.* dosage, timing, frequency)**	**Inclusion criteria: metformin; no dosage, timing, and frequency limit Exclusion criteria: combined with other anti-diabetic medication**	
22.	**Outcome measures**	**Inclusion criteria: all tumour-related outcomes Exclusion criteria: none**	
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: none Exclusion criteria: none**	
26.	**Sort and prioritize your exclusion**	**Phase: screening based title/abstract**	

	criteria per selection phase	**Excluded:** **1.not primary study** **2. not an in vivo animal study** **3. not disease of interest (HCC),** **4. not right intervention(metformin)** **Phase: only screening full-text** **Excluded:** **1.not a primary study** **2.not an in vivo animal study** **3.not disease of interest (HCC)** **4. no metformin**	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
27.	**Study ID (*e.g.* authors, year)**	**Authors, year**	
28.	**Study design characteristics (*e.g.* experimental groups, number of animals)**	**Description of control group, method of HCC induction. Reference, language, species/strain, gender, control group, n(c)/n (exp), method animal model induction, timing**	
29.	**Animal model characteristics (*e.g.* species, gender, disease induction)**	**Animal species/strain, gender, HCC induction**	
30.	**Intervention characteristics (*e.g.* intervention, timing, duration)**	**Intervention (drug name), timing, duration, route of administration**	
31.	**Outcome measures**	**tumour volume, weight, size and number**	
32.	**Other (*e.g.* drop-outs)**	**Number of animals excluded from statistical analysis, reason for excluding animals**	
	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]** **☐By use of SYRCLE's Risk of Bias tool, adapted as follows:** **☐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)**	**continuous: Tumour size, volume, weight;** **dichotomous: incidence of tumour** **counts: tumour number**	
36.	**Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting**	**1) Extract data from text or tables** **2) Extract data from figures (digital screen ruler)** **3) Contact authors for data not presented in paper**	

	authors)		
	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**	
38.	**How the decision as to whether a meta-analysis will be performed will be made**	**If the studies are sufficiently comparable (with regard to design etc.), outcome data will be pooled. Subgroup analyses will only be performed, if the overall meta-analysis contains a minimum of 4 studies.**	
	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)**	**mean difference & standard mean difference**	
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)**	**-study design -animal species/strain -type of HCC induction - dose/rout/timing/duration of administration - outcome measures**	
43.	**The method for assessment of publication bias**	**Funnel plot**	
44.	**Any sensitivity analyses you propose to perform**	**Effect of possible interactions by species and quality**	

Final approval by (names, affiliations):

Date: