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	Safety of using immortalized cell lines as treatment cell therapy in animal models for kidney diseases	
2.	Authors (names, affiliations, contributions)	Milos Mihaljovic* (PhD student) (Search strategy, in-exclusion criteria, data extraction, quality assessment, data-analysis, writing manuscript, meta-analysis (If feasible), writing manuscript) Thom van der Made* (master student) (Search strategy, in-exclusion criteria, data extraction, quality assessment, data-analysis) Dr. Rob de Vries‡ (support for structured systematic review , methodological support, Read and approved final version of the report;) Dr. Kim Wever ‡ (support for structured systematic review , methodological support, Read and approved final version of the report;) Dr. Roos Masereeuw* (support, manuscript review from clinical perspective, Read and approved final version of the report;) * Department of Pharmacology-Toxicology, Radboud Institute for Molecular Life Sciences (RIMLS), Nijmegen, The Netherlands. ‡ SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) at Central Animal Laboratory, Radboud University Medical Center, Nijmegen, the Netherlands	
3.	Other contributors (names, affiliations, contributions)		
4.	Contact person + e-mail address	Thom van der Made, thomvander.made@student.ru.nl	
5.	Funding sources/sponsors	-	
6.	Conflicts of interest	none	
7.	Date and location of protocol registration	-	
8.	Registration number (if applicable)	NA	
9.	Stage of review at time of registration	Searches performed, selection of studies in process	
	B. Objectives		

	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	The number of patients developing kidney diseases is growing drastically. For example the number of patients developing Chronic Kindey Disease (CKD) is reaching epidemic proportions (Meghuid El Nahas et al 2005). The current standard treatment option for CKD is dialysis, which remains suboptimal with high levels of morbidity and mortality. This therapy is very intensive as well and it does not cure the kidney disease. Other possible options, such as cell-based therapies, have gained a lot of attention in the last decade which can be illustrated by an example: the bioengineered kidney. Researchers worldwide have pursued the development of a bioartificial kidney, as this will have many benefits above the current standard care of kidney diseases. The bioartificial kidney would provide sufficient and continuous clearance of accumulating waste products and fluid balance without the need for hemodialysis, it can solve the organ scarcity problem, etc (Jansen et al 2014). Another example are the induced pluripotent stem cells (iPSC), which are an exciting field of science and make striking progresses in treating several diseases. Those therapies have proven to be a promising clinical approach for several pathological conditions and may represent a valuable tool as a therapeutic strategy. They are currently the focus of preclinical studies. Cell-based therapy involves replacing and transfecting cells to help protect against the disease. Pre-clinical studies have demonstrated beneficial effects after injection with various cell populations. Among these cell populations are immortalized cell lines such as the conditionally immortalized proximal tubule epithelial cells (ciPTEC) and HeLa cells. Immortalized cell lines have the characteristics that they grow and divide indefinitely *in vitro* and *in vivo* for as long as the correct culture conditions are maintained. Growth properties have been altered by transfection with viral vectors (transformation by infection with viral vectors). Vectors based on gammaretrovirus, lentivirus, adenovirus (AdV), adeno-associated virus (AAV) and herpes simplex virus (HSV) are among the most widely used viral vectors in current gene therapy studies[1]. However, as numerous clinical trials have proved the effectiveness of cell-based therapy, it is unclear what the risks are of these cell lines for future clinical use. Can an immune response occur? Is there a risk to develop a tumor due to characteristics of the cell lines? Ferreira et al. suggested that AAV-based vectors for gene therapy can	

		trigger the innate and adaptive immune system (Ferreira et al. (2014). A case report by Hacein-Bey-Abina et al showed us that there is a risk to develop a tumour after successful gene-therapy (Hacein-Bey-Abina et al 2003). This review will focus on the safety of these immortalized cell lines in animal models and clinical studies.	
	Research question		
11.	Specify the disease/health problem of interest	Safety of immortalized cell lines as treatment therapy for kidney disease	
12.	Specify the population/species studied	Animal models	
13.	Specify the intervention/exposure	administration of immortalized cells	
14.	Specify the control population	-	
15.	Specify the outcome measures	Outcome measures related to harmful effects including; mortality, tumour development, immune response (TNF-a, IL-6, IL-8), morphology and signs of toxicity or unusual behavior, organ specificity.	
16.	State your research question (based on items 11-15)	What is the current evidence for the safety of cell therapy using immortalized cell lines in animal models of kidney disease	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	XMEDLINE via PubMed ☐Web of Science ☐SCOPUS XEMBASE ☐Other, namely: Cochrane Controlled Trials Register (CENTRAL) ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide [1] and animal search filters [2, 3])	A search strategy composed of three components will be developed: * Animal models * Cell-based therapy (immortalized cells) *Kidney disease For "cell-based therapy" ,the thesaurus functions of Pubmed and EMBASE (MeSH database and EMTREE) were used to identify all indexation terms for these search components. Additional synonyms and search terms, also for non-indexed articles, were identified with the help of SYRCLE. To detect all animal studies in Pubmed and EMBASE, the animal search filter (available from SYRCLE) will be used. The search strategy for the kidney disease will be partly derived from a publication by Kim Wever et al. (2012)	

19.	Identify other sources for study identification	Reference lists of included studies ☐Books X Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	Articles in the reference list of relevant reviews will be screened on title; if potentially relevant the original article will be redeemed via PubMed or EMBASE, and screened for abstract (and if relevant the full text).	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	First selection phase: pre-screening on title and abstract. Second selection phase: screening of full text of the articles selected in the first phase.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Pre-screening will be done by two authors? (TvdM,MM). As a couple, two reviewers independently screen the same subset of titles and abstracts and then compare their findings via EROS software. If discrepancies occur, they consult a third reviewer(RdV). Once selected, full screening of the selected studies will be done independently by two authors. If discrepancies occur, they consult a third reviewer.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	*Inclusion criteria*: animal intervention studies (primary studies). *Exclusion criteria*: non-primary studies (reviews, conference proceedings, commentary).	
24.	Type of animals/population (*e.g.* age, gender, disease model)	all animal models for kidney disease	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	cell therapy using immortalized cell lines (induction via viral vector)	
26.	Outcome measures	Inclusion criteria: Reported harmful effects including mortality, tumor development, immune response (TNF-a, IL-6, IL-8), morphology, signs of toxicity, unusual behavior or organ specificity. (does not necessarily has to be primary outcome measurement) Exclusion criteria: Did not report safety data	
27.	Language restrictions	No restriction on languages	
28.	Publication date restrictions	No restriction on publication date	
29.	Other	Inclusion criteria: - Exclusion criteria: duplicate papers	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: pre-screening on title/abstract, exclusion if: 1. Not a primary study (e.g. reviews) 2. Not administered cells 3. Not kidney disease 4. SCID mouse used	

		5. No animal model Selection phase full text screening: 1. Not administered immortalized cells 2. Not reported safety data	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Author, year	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	• Experimental groups (incl type of controls) • Number of animals in each experimental group • Duration of follow up • Outcome measures • Timing of data collection	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	species, strain, age, gender, weight	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	• How was the cell line produced/ which cell line was used (which vector) • Dose • Duration of treatment • Type of injection	
35.	Outcome measures	• How was outcome measured • Were outcome assessors blinded • Was outcome measured on same time-point for all experimental groups	
36.	Other (*e.g.* drop-outs)	• Complications/safety aspects rate/unpredicted outcomes +cause (if known) • Therapy failure rate + reasons	
	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 researchers will assess risk of bias (TvdM,MM). A third reviewer will be consulted if discrepancies occur (RdV)	
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)	X 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 criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define	Mortality – dichotomous	

	the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Tumour development – continuous Immune response – continuous Morphology – continuous Signs of toxicity or unusual behavior - continuous Organ specificity - dichotomous	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	First, data will be extracted from the graphs/tables in the results sections of the articles. If necessary, authors will be contacted to retrieve information that could not be found in the article.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Two researchers will extract data (TvdM,MM). A third reviewer will be consulted if discrepancies occur (RdV)	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Initially, a descriptive summary of the safety outcome measurements will be written. Depending on the comparability of outcome measures, the quality and amount of available evidence identified in the literature a meta-analysis will be performed (+ subgroup- analysis).	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Outcome data will be pooled. A meta-analysis will be considered if 5 or more studies can be included. Subgroup analysis will be performed to explain heterogeneity between these studies.	
	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)	Continuous variables – standardized mean difference = difference in mean between treatment and control group divided by the pooled standard deviations. Categorical variables – risk ratio.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	The Random effects model will be used as this model allows us to account for differences in study design, animal models and housing conditions between studies . Effect size will be displayed in a forest plot.	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	For this systematic review I^2 will be used, this method describes the amount of the total variation that is due to between study variation. Heterogeneity was considered low, moderate or high at 25, 50 and 75% (Higgins et al., 2003)	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	• model-related (species, gender, timing of therapy , place of injection, dose and duration of treatment) • Type of immortalized cells (cell type, -condition, -origin, administration route and regime of therapy)	
48.	Any sensitivity analyses you propose to perform	-	

49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	-	
50.	The method for assessment of publication bias	Using RevMan 5.3 software (Cochrane informatics & knowledge management dept.), a funnel plot will be created to assess publication bias	
Final approval by (names, affiliations): Milos Mihaljovic , Rob de Vries, Kim Wever		Date: 29/07/2015	