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Are cell-based therapies for kidney disease safe? A systematic review of preclinical evidence

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ABSTRACT

The number of individuals affected by acute kidney injury (AKI) and chronic kidney disease (CKD) is constantly rising. In light of the limited availability of treatment options and their relative inefficacy, cell based therapeutic modalities have been studied. However, not many efforts are put into safety evaluation of such applications. The aim of this study was to review the existing published literature on adverse events reported in studies with genetically modified cells for treatment of kidney disease.

A systematic review was conducted by searching PubMed and EMBASE for relevant articles published until June 2018. The search results were screened and relevant articles selected using pre-defined criteria, by two researchers independently. After initial screening of 6894 abstracts, a total number of 97 preclinical studies was finally included for full assessment. Of these, 61 (63%) presented an inappropriate study design for the evaluation of safety parameters. Only 4 studies (4%) had the optimal study design, while 32 (33%) showed sub-optimal study design with either direct or indirect evidence of adverse events. The high heterogeneity of studies included regarding cell type and number, genetic modification, administration route, and kidney disease model applied, combined with the consistent lack of appropriate control groups, makes a reliable safety evaluation of kidney cell-based therapies impossible. Only a limited number of relevant studies included looked into essential safety-related outcomes, such as inflammatory (48%), tumorigenic and teratogenic potential (12%), cell biodistribution (82%), microbiological safety with respect to microorganism contamination and latent viruses' reactivation (1%), as well as overall well-being and animal survival (19%). In conclusion, for benign cell-based therapies, well-designed pre-clinical studies, including all control groups required and good manufacturing processes securing safety, need to be done early in development. Preferably, this should be performed side by side with efficacy evaluation and according to the official guidelines of leading health organizations.

Abbreviations: AKI, Acute kidney injury; CKD, Chronic kidney disease; ESRD, End stage renal disease; MSC, Mesenchymal stem cell; EPC, Endothelial progenitor cell; IGF-1, Insulin-like growth factor 1; HGF, Hepatocyte growth factor; EPO, Erythropoietin; CXCR, CXC chemokine receptor; SAA1, Serum Amyloid A1; iPS, Induced pluripotent stem cell; TGF-β, Transforming growth factor beta; α-SMA, Alpha smooth muscle actin; UUO, Unilateral ureter obstruction; I/R, Ischemia-reperfusion; PKD, Polycystic Kidney Disease; AF-MSC, Amniotic fluid-derived mesenchymal stem cell; hucMSC, Human-umbilical cord-derived mesenchymal stem cell; ADMSC, Adipose-derived mesenchymal stem cell; MRPC, Mouse renal progenitor cell; HSPC, Hematopoietic stem and progenitor cell; BM-MSC, Bone marrow-derived mesenchymal stem cell; BMDC, Bone marrow-derived cell; SRC, Selected renal cells; NRK-52E, Rat epithelial kidney cell line; rKS56, Proximal tubule S3 segment-derived renal progenitor-like cell line; EC, Endothelial cell; hRPTEC, Human renal proximal tubular epithelial cell; mRNA, Messenger RNA; GFP, Green fluorescent protein; BLI, Bioluminescence imaging; IHC, Immunohistochemistry; FISH, Fluorescence in situ hybridization; ISH, In situ hybridization; SRY, Sex-determining region; vWF, Von Willebrand factor; RFP, Red fluorescent protein; siRNA, short interfering RNA; BTD, Bioartificial tubule device; LPS, Lipopolysaccharide; MHC, Major histocompatibility complex.

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Contents

1. Introduction

Renal insufficiency represents an important health problem and there is a great need for development of new treatment options that could improve quality of life of kidney patients, but also reduce the global social and economic burden on the healthcare system (Levey et al., 2007). More awareness needs to be raised for prevention of acute kidney injury (AKI) (Lewington, Cerda, & Mehta, 2013) to reduce the mortality and the risk of developing chronic kidney disease (CKD). Also, more effort has to be put into improving the treatment of CKD itself and its progression towards end stage renal disease (ESRD) (Hill et al., 2016; Levey & Coresh, 2012). For ESRD, the preferred treatment option is kidney transplantation, but application is limited by donor organ shortage. Moreover, kidney transplantation is not an ideal treatment due to complications of immunosuppressive therapy. Consequently, patients with advanced age and those with extensive comorbidity are not eligible for transplantation. Therefore, many patients with ESRD are dependent on treatment with hemodialysis (Ortiz, et al., 2014). Despite many technological developments and advancements introduced in the field of dialysis in the past few decades, noticeable improvements regarding clinical outcomes, in particular patient survival, are very limited (Lameire, Van Biesen, & Vanholder, 2009). This is partly explained by the fact that dialysis does not efficiently remove metabolic waste products, leading to their accumulation. These so called uremic toxins have been associated with the development of other co-morbidities over time, especially cardiovascular disease, which remains the main cause of death within ESRD populations (Go, Chertow, Fan, McCulloch, & Hsu, 2004; Vanholder et al., 2008; Weiner et al., 2012). In addition, the treatment sessions are cumbersome for patients, reducing noticeably their quality of life (Jaar, Chang, & Plantinga, 2013; Jhamb, Weisbord, Steel, & Unruh, 2008), further indicating the need for alternative treatment strategies.

Tissue engineering and regenerative medicine embody extremely promising innovative strategies that could improve or replace functions of damaged organs, including the kidney, or even repair and regenerate them (Zambon et al., 2014). One of the recent developments in the field of tissue engineering includes the bioartificial kidney, comprised of viable epithelial cells of either allogeneic or xenogeneic origin, to make use of their transport machinery for a more efficient excretion of waste molecules (Humes et al., 2004; Humes, Buffington, MacKay, Funke, & Weitzel, 1999; Jansen et al., 2016; Takahashi et al., 2013). On the other hand, regenerative medicine related approaches might make use of acellular components, such as synthetic biomaterials and scaffolds, or decellularized kidneys that would maintain the complex 3D organization of extracellular matrix (ECM), thus allowing the optimal growth and differentiation of cells, in particular stem cells (Bonandrini et al., 2014; Little & Kairath, 2016; Orlando et al., 2013; Zambon et al., 2014). Alternatively, the stem cells could also be used directly for transplantation, without using scaffold materials, completely relaying on their regenerative capacities, including beneficial paracrine effects (Little & Kairath, 2016).

The most frequently used cell types for cell-based CKD treatment are mesenchymal stem cells (MSC), bone marrow cells (BMC), endothelial progenitor cells (EPC) and hematopoietic stem cells (HSC) (Papazova et al., 2015). However, other cells types have also been studied for their potential to treat kidney disease, such as primary kidney cells (Humes, Weitzel, & Fissell, 2004; Takahashi et al., 2013), cell lines (Kelly, Kluve-Beckerman, Zhang, & Dominguez, 2010) or cells genetically modified to overexpress certain proteins with therapeutic effects, such as insulin-like growth factor 1 (IGF-1) (Imberti et al., 2007), hepatocyte growth factor (HGF) (Chen et al., 2011), erythropoietin (EPO) (Kucic et al., 2008), CXC chemokine receptor type 4 (CXCR4) (Gheisari et al., 2012; N. Liu, Patzak, & Zhang, 2013), serum amyloid A1 (SAA1) (Kelly et al., 2010; Kelly et al., 2013; Kelly, Zhang, Wang, Zhang, & Dominguez, 2012), kallikrein (Hagiwara, Shen, Chao, & Chao, 2008), vascular endothelial growth factor (VEGF) (Togel, Zhang, Hu, & Westenfelder, 2009), and bone morphogenetic protein 7 (BMP-7) (Zhen-Qiang et al., 2012). In addition, recent advances in the field of induced pluripotent stem cells (iPS) as cell-based therapies for various pathologies, make them a very promising clinical approach and a valuable therapeutic tool for kidney failure as well (Takasato et al., 2015; Toyohara et al., 2015).

In various animal models of CKD, many of these treatment options were shown to be beneficial, as evaluated by improvement of several histological outcomes (glomerulosclerosis and tubular interstitial fibrosis), as well as functional parameters (glomerular filtration rate (GFR), blood pressure, urinary protein, plasma urea, plasma creatinine). Experimental models of CKD included ischemia-reperfusion injury, diabetic nephropathy, subtotal nephrectomy, hypertension or drug induced kidney disease (Papazova et al., 2015).

Despite these promising findings of cell-based therapy efficacy for CKD and ESRD, most studies have focused primarily on functional readouts and improvement of clinical parameters. An often neglected aspect and concern encountered when developing and improving cell-based therapies, is safety of the clinical application and especially the long-term effects.

Several safety issues related to cell-based therapies should be addressed prior to clinical application (European Medicines Agency, 2008; Food and Drug Administration, 2013). According to the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), among the most relevant issues are immunogenic, immunotoxic and inflammatory effects, especially in case of allogeneic and xenogeneic cells. Furthermore, immortalized or cells modified and transduced with retro- or lentiviral vectors are of particular concern given the possibility of release of vectors or plasmids. In addition, such modifications could significantly affect cell morphology and behavior, strongly associated with transformed phenotype and oncogenic and tumorigenic potential. Presence of microbial agents is another not negligible issue to be taken into consideration when evaluating the safety of a specific cell type for clinical applications. A systematic review of the evidence on safety of genetically modified cells in vivo would provide important information for future studies aiming to assess possible therapeutic effects and safety of cell-based therapies for kidney disease. Since no such synthesis of evidence currently exists, we have performed a systematic review of all published pre-clinical evidence on the safety of immortalized and genetically modified cells in animal models of kidney

disease. We investigated *a)* whether studies using genetically modified cells in animal models of kidney disease were set up to identify adverse outcomes or safety concerns and *b)* which safety concerns or adverse outcomes were reported.

2. Analysis

2.1. Review protocol and amendments

The review methodology was pre-specified in a review protocol and registered on http://www.syrcle.nl (see also (de Vries et al., 2015)). We made the following amendments to the protocol: the review has been extended to include all genetically modified cells in addition to immortalized cell lines. The review question therefore is: 'What is the current evidence for the safety of cell therapy using immortalized and genetically modified cells in animal models of kidney disease?'

2.2. Literature search

A systematic review was performed of all available studies reporting safety and adverse effects evaluation of cell-based therapies, in particular immortalized and genetically modified cells, in various animal models of kidney disease. A systematic literature search for articles published up to June 2018, was performed in PubMed and EMBASE. The full search strategies are included in Supplementary material S1 and involved the following components: "cell and tissue based therapy", "kidney disease", and "animals". All articles obtained by this search were evaluated by two independent researchers (M.M. being the first reviewer in all cases and K.E.W. or T.K. vd M. being the second reviewer) based on title and abstract, according to predefined inclusion and exclusion criteria. If abstracts were not available, or not informative enough, the full-text article was screened. If full-text articles were not available or accessible, corresponding authors were contacted via e-mail with a request to supply the full text. In case of discrepancies between the two reviewers, a third investigator (R.M.) was involved in the screening and discussion in order to reach a decision regarding inclusion. No language restrictions were applied. If needed, non-English articles were translated by native speaking scientists.

2.3. Inclusion and exclusion criteria

Articles were included if they were primary studies presenting unique data on in vivo experiments in which animals were treated with genetically modified cells to treat renal disease, and any outcome related to safety or adverse events was reported.

Articles that met at least one of the following criteria during the title and abstract screening phase were excluded: (1) there was no kidney disease, (2) there was no cell therapy intervention, (3) the study was not performed in animals in vivo, (4) immunodeficient animals were used, or (5) the study was not a primary study. After screening based on title and abstract, all included articles were subjected to full-text screening with additional exclusion criteria: (6) there was no administration of genetically modified or immortalized cells (7) there were no safety related outcomes measured or indicated, or (8) the full-text article could not be obtained.

2.4. Data extraction and analysis

The following study characteristics were extracted from all articles included: animal species, strain, age, sex, weight, kidney disease model, induction of kidney disease, cell type used for therapeutic intervention, cell origin, type of genetic modification of cells, number of cells administered, and administration route. Bibliographic data, such as author and year of publication, were also registered. The following outcomes related to safety and adverse effects were extracted: inflammatory and immune related markers (gene and protein cytokine levels, inflammatory cell infiltration), renal fibrosis (interstitial fibrosis, total collagen, gene and protein expression of transforming growth factor beta (TGF-β), alpha-smooth muscle actin (α-SMA), platelet-derived growth factor (PDGF), type I collagen and matrix metalloproteinases), apoptosis (TUNEL, caspase 3 activity and expression, Bax and Bcl2 expression), organ and tissue distribution of cells (kidneys and other distant organs), tumor and teratoma formation, and overall survival of animals.

2.5. Assessment of methodological quality

Methodological quality of the included studies was determined using SYRCLE's risk of bias tool (Hooijmans et al., 2014) with addition of reporting items as used in previous studies (Jonker, Menting, Warle, Ritskes-Hoitinga, & Wever, 2016; Wever et al., 2015). SYRCLE's risk of bias tool, which aims to establish consistency when assessing internal validity of animal intervention studies, covers five important domains: selection bias, performance bias, detection bias, attrition bias, reporting bias and other biases (Hooijmans et al., 2014). To assess selection bias due to differences in baseline characteristics between groups, we assessed whether groups were of equal strain, sex and weight or age. Risk of detection bias was assessed separately for all outcomes, versus for histological outcomes only. Risk of bias due to selective outcome reporting was not assessed, since none of the included studies referred to a prospectively registered study protocol, which would allow a comparison between predefined and actually reported outcomes.

3. Results

Due to the high heterogeneity in study characteristics and in reporting of outcomes among all articles included, a meta-analysis was not considered to be sensible. Therefore, a narrative synthesis of available data was performed.

3.1. Study selection and characteristics

The electronic search strategy retrieved 1665 articles from PubMed and 5228 articles from EMBASE. On one occasion an author provided a publication that did not appear in the electronic search (1 article via author). Of these, 5682 were unique and evaluated for inclusion based on title and abstract. In total, 531 articles met the inclusion criteria and were screened as full-text. After full-text assessment, 97 studies were included in this systematic review (Fig. 1).

Study characteristics of all articles included are summarized in Tables 1 and 2, reporting details of animal and kidney disease models, as well as the cell therapy used, and outcome measures related to safety and adverse events. There was considerable variation in the characteristics of the animals and the renal disease models employed. Fifty studies (51.5%) were performed in rats, forty-one (42.3%) in mice, two (2.1%) in goats, two (2.1%) in rabbits, one (1.0%) in pigs and one (1.0%) in dogs. Regarding the sex of the animals used, fifty-one studies (52.6%) used males, twenty-four (24.7%) used female animals, six (6.2%) used both sexes, while sixteen studies (16.5%) did not specify the sex of animals used. A plethora of renal disease models was used: forty-nine studies (50.5%) used an AKI model (either ischemia-reperfusion (I/R), cisplatin, gentamicin, nephrectomy, or sepsis-induced), twenty-one (21.7%) used a CKD model (either a subtotal nephrectomy or unilateral ureter obstruction (UUO)), seven (7.2%) a glomerulosclerosis model (both Alport syndrome and other models), two (2.1%) cystinosis, five (5.2%) a glomerulonephritis, six (6.2%) a diabetic nephropathy, two (2.1%) a polycystic kidney disease model, one (1.0%) a kidney transplantation model, one (1.0%) adriamycin-induced nephrotic syndrome model, one (1.0%) a radiation nephropathy model, one (1.0%) a mesangial sclerosis model and one (1.0%) study used both AKI and CKD models. Regarding the cell types used, a majority of studies (86; 88.7%) used various types of stem cells (see Table 2). Seven studies (7.2%) used

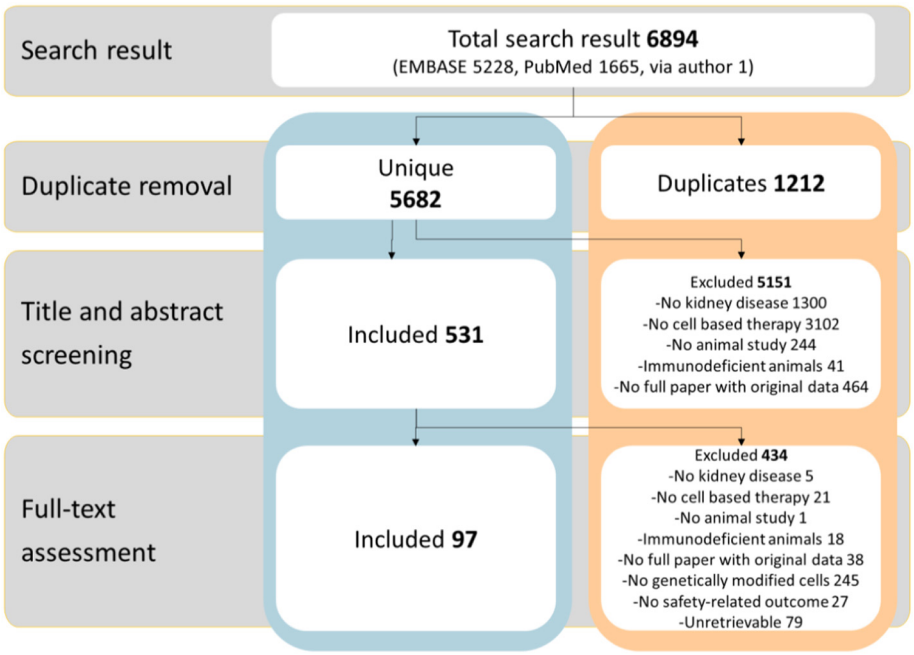


Fig. 1. Flow chart of study selection. Complete search strategy is described in Supplementary material S1. Inclusion and exclusion criteria used for articles selection are defined in the Analysis section.

primary cells (renal tubular cells, macrophages or endothelial cells) and only four studies (4.1%) used cell lines. The number of cells administered was also variable, with three studies (3.1%) using a cell number in the order of 10^8, seven (7.2%) in the order of 10^7, sixty-two (63.9%) in the order of 10^6, fourteen (14.4%) in the order of 10^5, two (2.1%) in the order of 10^4, six (6.2%) used two or more different amounts ranging from 10^3 to 10^7, while three (3.1%) studies did not specify the number of cells administered.

All articles included were subdivided in one of four categories based on study design, whether appropriate controls were used in order to determine side effects and risk factors, as well as whether such adverse effects were reported explicitly, and characteristics were explained. Description of all categories and categorized articles is shown in Fig. 2a-b. The optimal study design which would allow to determine both the efficacy and safety of a specific type of genetically modified cells is summarized in Fig. 2c.

The majority of studies (63%) did not have appropriate control groups nor did they report any adverse effects related to the cell therapy implemented to treat kidney disease. In 30% of the studies, a suboptimal study design was found, presenting indirect evidence of cell therapy safety, while specifically reporting adverse events during the course of the study. A total of seven studies had the suitable study design allowing the assessment and monitoring of adverse effects. Out of these seven, three studies (3%) applied genetically modified cells in healthy control animals but did not specifically evaluate adverse events, while four (4%) were optimally designed and did monitor and report the side effects and cell therapy-related problems.

3.2. Reporting quality and internal validity of studies

The results of the quality assessment are shown in Fig. 3. Our assessment of reporting of measures to reduce bias and key indicators of study quality (randomization, blinding, sample size calculation and conflict of interest statement) shows that reporting of these was very poor (Fig. 3a). Out of ninety-seven studies, only thirty-four (35%) reported randomization of the study at any level, and only one (1%) reported the method of randomization used. Forty-three (44%) studies reported blinding of the study at any level, which in all cases concerned blinding of the outcome measure histology only. Only one study reported a sample size calculation. A statement regarding conflict of interest was reported more frequently, i.e. by seventy-six (78%) studies. As a consequence of poor reporting of measures to reduce bias, our assessment of risk of bias in the included studies yielded mostly unclear risks of selection, performance and detection bias (Fig. 3b). Out of ninety-seven studies, fifty-six (58%) reported sufficient data on baseline characteristics of the animals (strain, sex and weight or age) to assess that these were similar between groups, which suggests a low risk of bias. Regarding attrition bias, fourteen studies (14%) were assessed as high risk of bias due to unexplained drop-outs. In seventy-one studies (73%) the risk of bias was unclear, because the number of drop-outs could not be assessed or the number of animals per group was not clear. Twelve (12%) studies were assessed as low risk of bias because there were no drop-outs, or the reason of death of experimental animals in each group was specified. Regarding other risks of bias, three studies (3%) exhibited high risk of bias because authors were shareholder of a financially involved company without reporting conflict of interest, or authors had patents pending. The remaining studies were assessed as either low risk of bias (72; 74%), because they explicitly reported no conflict of interest and appeared to be free of other risks of bias, or unclear risk of bias because a conflict of interest statement was lacking (22; 23%).

3.3. Kidney disease cell therapy-related adverse effects

Several risk factors and side effects of cell therapy that are generally recognized (European Medicines Agency, 2012; Herberts, Kwa, & Hermsen, 2011) (schematically shown in Fig. 4) were considered in all included kidney disease animal studies. Most of the risk factors and side effects considered were related to stem cell-based therapies, given the fact that the majority of studies used stem cells. Nonetheless, the information derived can be easily extrapolated to studies with other cell types, especially genetically modified cells. However, the description of various risk factors, including immunogenicity, tumorigenic effects, teratoma formation, biodistribution, microbial contaminations and overall well-being and survival, varied between studies depending on the cell type or genetic manipulation of the cells. Here, we discuss in more detail their relevance in perspective of the cells used in the selected articles.

Table 1
Study characteristics: Animals and kidney disease models.

Author and Year	Species	Strain	Age (weeks)	Sex (M/F)	Weight (g)	Kidney disease model	Induction of kidney disease
Bai et al., 2018	Mouse	C57BL/6	NR	NR	20–25	AKI (I/R)	Renal pedicles clamping for 35 min
Bajwa et al., 2016	Mouse	C57BL/6J, Balb/c	8–12	M	NR	AKI (I/R)	Renal pedicles clamping for 26–28 min
Bataille et al., 2016	Mouse	C57BL/6J	8	F	16–21	AKI	Intraperitoneal injection of cisplatin (20 mg/kg)
Baulier et al., 2014	Pig	NR	12	NR	NR	Kidney transplantation	Renal pedicle clamping for 60 min followed by kidney removal, preservation and transplantation 6 days later
Bian et al., 2014	Rat	Sprague Dawley	Adult	M	200–250	CKD	Subtotal 5/6 nephrectomy
Bian et al. (2014)	Rat	Sprague Dawley	NR	M	200–250	CKD	Subtotal 5/6 nephrectomy
Caldas et al., 2017	Rat	Wistar	Adult	F	NR	CKD	Subtotal 5/6 nephrectomy
Chen et al., 2008	Mouse	FVB/NJ	8–20	M	NR	AKI (I/R)	Unilateral or bilateral renal pedicle clamping for 30 min
Chen et al., 2011	Rat	Sprague Dawley	Adult	F	200–250	AKI (I/R)	Bilateral renal pedicle clamping for 60 min
Chen et al., 2015	Mouse	C57BL/6	8–10	F	20–25	CKD	Subtotal 5/6 nephrectomy
Du et al., 2016	Mouse	C57BL/6	8–10	M	NR	Glomerulonephritis	Nephrotoxic serum induced glomerulonephritis
Eliopoulos et al., 2011	Mouse	BALB/c	8–10	F	NR	AKI	Subcutaneous injection of cisplatin (14.7 mg/kg)
Ezquer et al., 2015	Mouse	C57BL/6	8	M	NR	Diabetic nephropathy	Intraperitoneal injection of streptozotocin (200 mg/kg)
Feng et al., 2016	Mouse	FVB	Adult	NR	NR	AKI (I/R)	Left renal pedicle clamping for 40 min
Feng, Lu, Dai, Sheng, & Xu, 2018	Mouse	C57BL/6	8	M	NR	Diabetic nephropathy	C57BL/KsJ db/db obese transgenic rats
Ferenbach et al., 2010	Mouse	FVB/NJ	6–8	M	NR	AKI (I/R)	Left renal pedicle clamping for 20 min
Franchi et al., 2014	Rat	Sprague Dawley	6	F	NR	PKD	Rat PKD model
Furuichi et al., 2012	Mouse	C57BL/6J	6–8	M	NR	AKI (I/R)	Left renal artery and vein clamping for 45 min
Gao et al., 2012	Rat	Sprague Dawley	NR	M	180–200	AKI (I/R)	Renal pedicles clamping for 40 min
Geng et al., 2014	Mouse	C57BL/6	8–12	M	NR	AKI (rhabdomyolysis)	Glycerol administration (8 ml/kg body weight)
Gheisari et al., 2012	Mouse	BALB/c	8–12	M	20–25	AKI	Subcutaneous injection of cisplatin (18 mg/kg)
Golle et al., 2017	Rat	Sprague Dawley	NR	M	260–300	CKD	NR
Gregorini et al., 2016	Rat	Sprague Dawley	NR	M	250–300	CKD (UUO)	Right ureteral ligation
Guiteras et al., 2017	Mouse	C57BL/6	8	M	NR	CKD (UUO)	Left ureteral ligation
Guo, Ardito, Kashgarian, & Krause, 2006	Mouse	Wt1 +/− (FVB/N x C57Bl/6)	8	NR	NR	Mesangial sclerosis	Wt1 +/− transgenic mice with late onset of mesangial sclerosis
Hagiwara et al., 2008	Rat	Wistar	NR	M	NR	AKI (I/R)	Renal pedicles clamping for 40 min
Han et al., 2013	Mouse	C57BL/6	8	M	27–32	AKI (I/R)	Renal pedicles clamping for 30 min
Harrison et al., 2013	Mouse	C57BL/6	4–16	M/F	NR	Cystinosis	Cystinosis transgenic mouse model (CTNS−/−)
Huang et al., 2012	Rat	NR	NR	NR	NR	Glomerulonephritis	Administration of rabbit antibodies against glomerular basement membrane
Huuskes et al., 2014	Mouse	C57BL/6J	NR	M	20–25	CKD (UUO)	Left ureteral double ligation
Imberti et al., 2007	Mouse	C57BL/6J	8	F	NR	AKI	Subcutaneous injection of cisplatin (12.7 mg/kg)
Jia et al., 2016	Rat	Wistar	NR	M	80–100	CKD	2% adenine suspension (200 mg/(kg*d))
Jiang et al., 2015	Mouse	C57BL/6	NR	NR	NR	AKI (I/R)	Renal pedicles clamping for 25 min
Kankuri et al., 2015	Rat	Sprague Dawley	6–7	M	NR	AKI (I/R)	Renal pedicles clamping for 40 min
Katayama et al., 2008	Mouse	129 × 1/SvJ	3	F	NR	Alport syndrome (glomerulosclerosis)	Alport syndrome transgenic model
Katsuoka et al., 2015	Rat	Lewis	10–12	NR	NR	AKI	0.75% adenine diet supplementation for 4 weeks
Kelley et al., 2013	Rat	ZSF1	18	M/F	NR	Diabetic nephropathy	ZSF1 rat diabetic nephropathy model
Kelly et al., 2010	Rat	Sprague Dawley	NR	NR	250	AKI	Renal pedicles clamping for 30 min; gentamicin (100 $mg \cdot kg^{-1} \cdot day^{-1}$ twice daily for 7 days; cisplatin (7.5 mg/kg))
Kelly et al., 2012	Rat	Sprague Dawley	NR	F	150–200	AKI (I/R); CKD	Renal pedicles clamping for 50 min; intraperitoneal injection of cisplatin (1.5 mg/kg every other day, 3 doses)
Kelly et al., 2013	Rat	Sprague Dawley	10	F	NR	CKD	Renal pedicles clamping for 25 min in diabetic rat model
Kelly et al., 2015	Rat	PCK	6–10	F	NR	PKD, I/R	Rat PKD model, left renal pedicle clamping for 50 min
Kinomura et al., 2008	Rat	Sprague Dawley	NR	M	200–250	AKI	Intraperitoneal injection of cisplatin (6 mg/kg)
Kluth et al., 2001	Rat	Sprague Dawley	NR	M	200–250	Nephrotoxic nephritis	Rabbit nephrotoxic serum injection (1 ml/200 g)
Kucic et al., 2008	Mouse	C57BL/6	NR	F	NR	CKD	Electrocoagulation right kidney, followed by left kidney nephrectomy after 22 days
LeBleu et al., 2009	Mouse	C57BL/6, 129Sv	5–8	F	NR	Alport syndrome (glomerulosclerosis)	Alport syndrome transgenic model
Lee et al., 2012	Rat	Sprague Dawley	8	M	300	AKI (I/R)	Left renal artery clamping for 45 min

(continued on next page)

Table 1 (*continued*)

Author and Year	Species	Strain	Age (weeks)	Sex (M/F)	Weight (g)	Kidney disease model	Induction of kidney disease
Lee et al., 2017	Dog	Beagle	24–48	M	10.000–15.000	AKI	Intravenous injection of gentamicin (15 mg/kg) three times per day for two weeks followed by a final single injection of cisplatin (70 mg/m^2)
Li, Morioka, Uchiyama, & Oite, 2006	Rat	Sprague Dawley	10	NR	NR	Glomerulosclerosis	Intravenous injection of anti-rat Thy1 monoclonal antibody (1 mg/kg/body weight)
Li, Deane, Campanale, Bertram, and Ricardo (2006)	Mouse	BALB/c	6	M	20–25	Adriamycin induced nephrosis	Intravenous adriamycin injection (10.5 mg/kg) 12 weeks after cell transplantation
Li et al., 2012	Mouse	B6-Ly5.2	6-8	F	NR	AKI (I/R)	Left renal pedicle clamping for 35 min
Li et al., 2014	Mouse	129/svj	8	NR	NR	Glomerulonephritis	Rabbit anti-mouse glomerular basement membrane serum
Li et al., 2015	Rat	Sprague Dawley	8	M	3.000–3.500	AKI (I/R)	Left renal pedicle clamping for 45 min
Lin et al., 2003	Mouse	B6-Ly5.2/Cr	6	F	NR	AKI (I/R)	Left renal artery clamping for 15 min
Lira et al., 2017	Rat	Wistar	8	M	NR	Renovascular hypertension	Partial occlusion of left renal artery
Liu, Shen, Yang, & Liu, 2011	Rat	Sprague Dawley	6	F	140–160	CKD (UUO)	Left ureteral double ligation
Liu et al., 2013	Mouse	C57BL/6J	6–8	NR	NR	AKI (I/R)	Bilateral renal pedicles clamping for 30 min
Liu et al., 2015	Rat	Sprague Dawley	6–8	NR	150–200	AKI (I/R)	Renal pedicles clamping for 45 min
Liu et al., 2016	Rat	Sprague Dawley	NR	M/F	250–300	AKI (I/R)	Renal pedicles clamping for 40 min
Luo et al., 2014	Mouse	C57BL/6	NR	M	25–30	AKI (sepsis associated)	Cecal ligation and puncture (CLP)
Lv et al., 2014	Rat	Wistar	NR	F	200–250	Diabetic nephropathy	Intraperitoneal injection of streptozotocin (60 mg/kg)
Monteiro Carvalho Mori da Cunha et al., 2015	Rat	Wistar	12	M	200–300	AKI (I/R)	Renal pedicles clamping for 50 min
Mori da Cunha et al., 2017	Rat	Wistar	12	M	NR	AKI (I/R)	Renal pedicles clamping for 50 min
Ornellas et al. 2017	Rat	Wistar	14–16	F	NR	AKI (I/R)	Renal pedicles clamping for 60 min
Ozbek et al., 2015	Rat	Wistar	NR	M	250–300	CKD (UUO)	Left ureteral ligation
Pacurari et al., 2013	Rat	Sprague Dawley	11	M	NR	CKD	Subtotal 5/6 nephrectomy
Prodromidi et al., 2006	Mouse	C57BL/6	6–8	F	NR	Alport syndrome (glomerulosclerosis)	Alport syndrome transgenic model
Rampino et al., 2011	Rat	Sprague Dawley	NR	M	180–200	Glomerulosclerosis	Intravenous injection of anti-rat Thy1 monoclonal antibody (0.4 mg/rat)
Roudkenar et al., 2018	Rat	*Rattus norvegicus*	6	F	160–180	AKI	Intraperitoneal injection of cisplatin (3–16 mg/kg)
Ruan et al., 2013	Rabbit	Japanese white rabbits	12	NR	2.000–2.600	CKD (UUO)	Left ureteral ligation
Saito et al., 2012	Goat	Japanese Saanen	NR	M	32.000–50.000	AKI	Bilateral nephrectomy and LPS injection (5×10^5 IU/kg) for 2 h
Shuai et al., 2012	Rat	Sprague Dawley	NR	F	180–200	CKD	Subtotal 5/6 nephrectomy
Si, Liu, Li, & Wu, 2015	Rat	Sprague Dawley	8	M	180	AKI (I/R)	Renal pedicles clamping for 40 min
Song et al., 2017	Rat	Wistar	NR	M	350–400	CKD (UUO)	Left ureteral double ligation
Sugimoto et al., 2006	Mouse	C57BL/6	8	M/F	NR	Alport syndrome (glomerulosclerosis)	Alport syndrome transgenic model
Syres et al., 2009	Mouse	C57BL/6	8–16	M/F	NR	Cystinosis	Cystinosis transgenic mouse model (CTNS−/−)
Takahashi et al., 2013	Goat	Japanese Saanen	NR	M	NR	AKI	Bilateral nephrectomy, after 48 h LPS injection for 2 h during circulation
Tang et al., 2018	Mouse	C57BL/6	8	M	NR	Diabetic nephropathy	C57BL/KsJ db/db obese transgenic rat model
Tian et al., 2017	Rat	Sprague Dawley	8–10	M	180–200	AKI (I/R)	Right kidney removal, followed by left renal pedicle clamping for 45 min
Togel et al., 2008	Mouse	C57BL/6	Adult	M/F	20–25	AKI (I/R)	Renal pedicles clamping for 30 min
Togel, Cohen, et al., 2009	Rat	Sprague Dawley, F344	Adult	M	200–300	AKI (I/R)	Renal pedicles clamping for 58, 40 or 35 min
Togel, Zhang, et al. (2009)	Rat	Sprague Dawley	Adult	F	200–250	AKI (I/R)	Renal pedicles clamping for 48 min
Tsuda et al., 2010	Rat	Lewis	6	M	170–180	Glomerulonephritis	Intravenous injection of anti-rat Thy1 monoclonal antibody (0.5 mg/rat)
Tsuda et al., 2014	Rat	Lewis	10	M	230–250	AKI (I/R)	Renal pedicles clamping for 60 min
Uchida et al., 2017	Mouse	Balb/c, SCID	11–14	M	NR	Glomerulosclerosis	Intravenous injection of adriamycin (11.5 mg/kg)
van Koppen et al., 2012	Rat	Lewis	NR	M	NR	CKD	Subtotal 5/6 nephrectomy
van Koppen et al., 2015	Rat	Lewis	8	M	NR	CKD	Subtotal 5/6 nephrectomy
Wang et al., 2015	Rat	Sprague Dawley	Adult	M	190–220	Diabetic nephropathy	Intraperitoneal injection of streptozotocin (40 mg/kg)
Wang, Zhang, Zhuo, Wu, Xu, et al., 2016	Rat	Sprague Dawley	4	NR	250–300	AKI	Subcutaneous injection of mercuric chloride (0.75 mg/kg)
Wang, Zhang, Zhuo, Wu, Liu, et al., 2016	Rat	Sprague Dawley	6–8	NR	NR	Radiation nephropathy	Right kidney irradiation

Table 1 (*continued*)

Author and Year	Species	Strain	Age (weeks)	Sex (M/F)	Weight (g)	Kidney disease model	Induction of kidney disease
Wise et al., 2014	Mouse	C57BL/6J	6–8	M	NR	AKI (I/R)	Left renal pedicle clamping for 40 min or bilateral renal pedicles clamping for 25 min
Yamagishi et al., 2001	Mouse	DBA/2j	8	F	NR	CKD (UUO)	Right ureteral ligation
Yokote, Katsuoka, Yamada, Ohkido, & Yokoo, 2017	Rat	Lewis	10	M	NR	CKD	0.75% adenine for 2 weeks
Yuan, Wang, Chen, Zhou, & Han, 2017	Mouse	KM/NIH	NR	F	32–36	AKI (I/R)	Renal pedicles clamping for 60 min
Yuzeng et al., 2014	Mouse	C57BL/6	6	M	NR	AKI (I/R)	Bilateral renal pedicles clamping for 40 min
Zhen-Qiang et al., 2012	Rabbit	NR	NR	NR	NR	AKI (I/R)	Left renal artery and vein clamping for 60 min
Zhu et al., 2017	Mouse	C57BL/6	8–10	M	22–25	AKI (I/R)	Left renal pedicle clamping for 30 min
Zhuo et al., 2013	Rat	Sprague Dawley	8–10	M	250–300	AKI (I/R)	Right renal artery and vein clamping for 60 min

Sex (M/F) – Sex male or female; Weight (g) – Weight in grams. AKI – Acute Kidney Injury; CKD- Chronic Kidney Disease; I/R – Ischemia Reperfusion Injury; PKD – Polycystic Kidney Disease; UUO - Unilateral Ureteral Obstruction; LPS – Lipopolysaccharide; NR – Not Reported.

Table 2
Study characteristics: Cell therapy applied (cell type, origin, modification, number and administration route) and outcomes measured.

Author and Year	Cell type	Cell origin (species)	Type of genetic modification (Gene of interest)	Number of cells	Administration route	Outcomes
Bai et al., 2018	MSCs	Allogeneic	Transgenic mouse (RFP)	1×10^6	Intravenous	I, D
Bajwa et al., 2016	BMDCs	Allogeneic	Transgenic mouse (S1pr3 knock out)	5×10^6	Intravenous	D
Bataille et al., 2016	BMDCs	Allogeneic	Transgenic mouse (GFP)	1×10^6	Retro-orbital	F, A, D, S
Baulier et al., 2014	AF-MSCs	Autologous	Lentiviral transduction (GFP)	1×10^6/kg body weight	Intrarenal artery	I, F, D
Bian, Zhou, et al., 2014	MSCs	Allogeneic	Lentiviral transduction (GFP)	1×10^6	Intravenous	F, D
Bian, Zhang, et al. (2014)	MSCs	Allogeneic	Transgenic mouse (GFP)	1×10^7	Intravenous	F, D
Caldas et al., 2017	iPS	Allogeneic	Lentiviral transduction (Sox-17, Nanog, Oct-4)	0.5×10^6	Parenchymal (intrarenal)	I, F, D, T
Chen et al., 2008	4E cell line	Allogeneic	Transgenic mouse (Tie-2/GFP)	1×10^6	Intravenous	I, F, A, D
Chen et al., 2011	hucMSCs	Xenogeneic (human)	Adenoviral transduction (GFP, HGF)	1×10^6	Intracarotid artery	I, A, D, T
Chen et al., 2015	MKPCs	Allogeneic	Transgenic mouse (Myh9-targeted mutant)	2.5×10^5	Intravenous	I, F, D, S
Du et al., 2016	Macrophages; BMCs	Allogeneic	iPS-derived macropahges; BMCs from EGFP transgenic mice	1×10^6	Intravenous	I, F, D
Eliopoulos et al., 2011	MSCs	Allogeneic	Retroviral transduction (EPO)	5×10^6	Intraperitoneal	A, D, S
Ezquer et al., 2015	MSCs	Allogeneic	Transgenic mouse (GFP)	5×10^5	Intravenous	D
Feng et al., 2016	ADSCs	Allogeneic	Transgenic rat (GFP-Fluc)	1×10^6	Intrarenal (cortex)	I, F, A, D
Feng et al., 2018	AFSCs	Allogeneic	Adenoviral transduction (SIRT3)	3×10^6	Intrarenal	I, F, O, A
Ferenbach et al., 2010	Macrophages	Allogeneic	Adenoviral transduction (HO-1)	NR	Intravenous	I, A, D
Franchi et al., 2014	MSCs	Allogeneic	Transfection (Luciferase)	2.5×10^5	Intrarenal artery	F,D
Furuichi et al., 2012	ADMSCs	Allogeneic	Transgenic mouse (GFP)	1×10^5	Intravenous	I, F, D
Gao et al., 2012	ADMSCs	Allogeneic	Lentiviral transduction (Luciferase, RFP)	2×10^6	Intrarenal (cortex)	A, D
Geng et al., 2014	MSCs	Allogeneic	NR (RFP)	1×10^6	Intravenous	D
Gheisari et al., 2012	MSCs	Allogeneic	Lentiviral transduction (CXCR4, CXCR7, GFP)	5×10^5	Intravenous	A, D, S
Golle et al., 2017	BMDCs	Allogeneic	Transgenic rat (GFP)	3×10^7/week (for two weeks)	Intravenous	D
Gregorini et al., 2016	MSCs	Allogeneic	Transgenic mouse (GFP)	3×10^6	Intravenous	I, F, A, D
Guiteras et al., 2017	RAW 264.7 macrophages (cell line)	Allogeneic	Adenoviral transduction (NGAL)	1×10^6	Intravenous	I, F, O, D
Guo et al., 2006	BMCs	Allogeneic	Transgenic mouse (GFP)	2×10^7	Intrarenal	I, D, S
Hagiwara et al., 2008	MSCs	Allogeneic	Adenovirus transduction (GFP, kallikrein)	1×10^6	Intracarotid artery	I, A, D
Han et al., 2013	MRPC	Allogeneic	Transgenic mouse (GFP)	5×10^5	Intravenous	I, D, T
Harrison et al., 2013	HSPCs	Allogeneic	Lentiviral transduction (GFP, Luciferase, CTNS, CTNS-GFP)	1×10^6	Intravenous	I, D, T
Huang et al., 2012	BM-MSCs	Xenogeneic (human)	Adenoviral transduction (GDNF-GFP)	3×10^4 - 1×10^6	Intrarenal artery	I, D
Huuskes et al., 2014	BM-MSCs	Xenogeneic (human)	Lentiviral transduction (Luciferase, EGFP)	1×10^6	Intravenous	D
Imberti et al., 2007	MSCs	Allogeneic	Transfection (IGF1 siRNA)	2×10^5	Intravenous	D
Jia et al., 2016	BM-MSCs	Allogeneic	Transgenic rat (GFP)	1×10^6	Intravenous	D
Jiang et al., 2015	MSCs (Nestin +)	Allogeneic	Transgenic mouse (GFP)	2×10^6	Intravenous	F, A
Kankuri et al., 2015	BMSCs	Allogeneic	Transgenic rat (human angiotensinogen/renin)	6×10^6	Intravenous	I
Katayama et al., 2008	BMDCs	Allogeneic	Transgenic mouse (COL4A3 knock out)	1×10^7	Intravenous	F, S
Katsuoka et al., 2015	MSCs	Allogeneic	Transgenic rat (Luciferase)	1×10^6	Intravenous, intra-arterial	D

(*continued on next page*)

Table 2 (*continued*)

Author and Year	Cell type	Cell origin (species)	Type of genetic modification (Gene of interest)	Number of cells	Administration route	Outcomes
Kelley et al., 2013	SRCs	Allogeneic	Transgenic mouse (Tomato red fluorophore reporter)	2.5×10^6	Parenchymal (kidney)	I, F, D, S
Kelly et al., 2010	NRK-52E	Allogeneic	Transfection (SAA1.1, GFP, reporter pNF-kB-SEAP)	1×10^6	Intravenous	I, A, D
Kelly et al., 2012	Renal tubular cells	Allogeneic	Transfection (GFP, SIRT1, SAA1, BFP)	1×10^6	Intravenous	I, F, A, D, S
Kelly et al., 2013	Kidney tubular cells*	Allogeneic	Transfection (GFP, SAA1)	1×10^6	Intravenous	I, A, F, D
Kelly et al., 2015	Primary renal tubular cells	Allogeneic	Transfection (GFP, SAA1)	1×10^6	Intravenous	F, D
Kinomura et al., 2008	rKS56	Allogeneic	Transfection (β-galactosidase)	1×10^6/kidney	Subcapsular, intrarenal artery	A, D, T*
Kluth et al., 2001	NR8383 (rat alveolar macrophage cell line)	Allogeneic	Adenoviral transfection (IL-4, b-galactosidase)	6×10^6	Renal artery	I, D
Kucic et al., 2008	MSCs	Allogeneic	Retroviral transduction (EPO, IGF-1)	2×10^6 (in matrigel)	Subcutaneous	A
LeBleu et al., 2009	BMDCs	Allogeneic	Transgenic mouse (COL4A3 knock out, GFP)	1×10^6	Retro-orbital	S
Lee et al., 2012	iPS	Xenogeneic (mouse)	Retroviral transduction (Oct-4, Sox2, Klf4, EGFP)	5×10^4 - 50×10^6	Intrarenal artery	I, A, D, T, S
Lee et al., 2017	cUCB-MSCs	Allogeneic	Lentiviral transduction (GFP)	1×10^6	Intrarenal (corticomedullary junction)	D, S
Li, Morioka, et al., 2006	BMDCs	Allogeneic	Transgenic rat (EGFP)	1×10^8	Intravenous	I, D, S
Li, Deane, et al. (2006)	BMDCs	Allogeneic	Transgenic mouse (EGFP)	1×10^6	Intravenous	F, A, D
Li et al., 2012	HSPCs (Lin-)	Allogeneic	Transgenic mouse (CreKsp: R26R-EYFP)	5×10^6	Intravenous	A, D, T
Li et al., 2014	MSCs	Allogeneic	Lentiviral transduction (GSTM2, GFP)	1×10^6	Intravenous	I, O, A, D
Li et al., 2015	iPS-derived RPCs	NR	Transduction (GFP)	1×10^5	Parenchymal (cells/hydrogel suspension)	I, F, A, D, T
Lin et al., 2003	HSPCs (Lin-)	Allogeneic	Transgenic mouse (β-galactosidase)	2×10^3 (from Rosa26) + 2×10^5 (Lin- BM cells)	Intravenous	I, A, D
Lira et al., 2017	MSCs	Allogeneic	Transgenic rat (EGFP)	1×10^6	Subcapsular	D
Liu et al., 2011	MSCs	Allogeneic	Adenoviral transduction (GFP, HGF)	1×10^6	Intravenous	F, D
Liu et al., 2013	BM-MSCs	Allogeneic	Lentiviral transduction (CXCR4, GFP)	2×10^6	Intravenous	A, D
Liu et al., 2015	BMSCs	Allogeneic	Lentiviral transduction (HO-1, EGFP)	2×10^6	Renal artery	A
Liu et al., 2016	AF-MSCs	Xenogeneic (human)	Transfection (EPO)	2×10^6	Parenchymal (kidney)	A, D
Luo et al., 2014	BM-MSCs	Allogeneic	Transgenic mouse (RFP)	1×10^6	Intravenous	I, D, S
Lv et al., 2014	MSCs	Allogeneic	Lentiviral transduction (GFP)	2×10^6	Intravenous	F, D
Monteiro Carvalho Mori da Cunha et al., 2015	hAFSCs	Xenogeneic (human)	Lentiviral transduction (b-galactosidase)	1×10^6	Intra-arterial	D
Mori da Cunha et al., 2017	hAFSCs	Xenogeneic (human)	Lentiviral transduction (VEGF and truncated CD34)	1×10^6	Aorta	F, O
Ornellas et al. 2017	BMMCs	Allogeneic	Transgenic mouse (GFP)	1×10^6 cells	Intravenous	D
Ozbek et al., 2015	MSCs	Allogeneic	Transfection (VEGF-GFP)	1×10^6 cells	Intravenous	I, F, O, D
Pacurari et al., 2013	EC	Allogeneic	Adenoviral vector (GFP)	1.5×10^6	Intravenous	D
Prodromidi et al., 2006	BMDCs	Allogeneic	Transgenic mouse (COL4A3 knock out)	10×10^6; 5×10^5 MSCs + 1×10^6 (Col4a3−/− cells)	Intravenous	F, D
Rampino et al., 2011	MSCs	Allogeneic	Transgenic rat (EGFP)	3×10^6	Intravenous	I, F, D
Roudkenar et al., 2017	MSCs	Allogeneic	Transfection (Lcn2 (lipocalin), GFP)	1.5×10^6	Intravenous	F, O, D, T, S
Ruan et al., 2013	Fibroblasts (induced)	Autologous	Lentiviral transduction (EGFP)	2×10^5	Intrarenal artery	I, F, D
Saito et al., 2012	hRPTEC	Xenogeneic (human)	Transfection (p16INK4a siRNA)	3×10^8 - 7×10^8	Extracorporeal circuit via jugular vein	I, S
Shuai et al., 2012	EPCs	Allogeneic	Transfection (TERT)	1×10^5	Intravenous	I, F, T
Si et al., 2015	MSCs	Allogeneic	Lentiviral transduction (TGF-b1, EGFP)	4×10^6	Intravenous	I, D
Song et al., 2017	ADMSCs	Allogeneic	Lentiviral transduction (GFP)	5×10^6	Intravenous	D
Sugimoto et al., 2006	BMCs	Allogeneic	Transgenic mouse (COL4A3 knock out, LacZ)	2×10^6 - 5×10^6 cells	Intravenous	F, D
Syres et al., 2009	BMDCs	Allogeneic	Transgenic mouse (GFP, Luciferase)	2×10^7 (knock out cells); 1×10^6 (MSCs)	Intravenous	D
Takahashi et al., 2013	hRPTEC	Xenogeneic (human)	Transfection (p16INK4a siRNA)	3×10^8 - 7×10^8	Extracorporeal circuit via jugular vein	I, S
Tang et al., 2018	ASCs	Allogeneic	Transfection (HIF-1α shRNA)	3×10^6	Intrarenal	I, F
Tian et al., 2017	USCs	Xenogeneic (human)	Lentiviral transduction (GFP)	1×10^5	Intrarenal (cortex)	I, F, A, D
Togel et al., 2008	MSCs	Allogeneic	Transfection (Luciferase)	1×10^5	Intravenous	A, D

Table 2 (*continued*)

Author and Year	Cell type	Cell origin (species)	Type of genetic modification (Gene of interest)	Number of cells	Administration route	Outcomes
Togel, Cohen, et al., 2009	MSCs	Allogeneic	Transgenic rat (hPAP)	5×10^5, 2×10^6 or 5×10^6/kg body weight	Intracarotid artery	F, T, S
Togel, Zhang, et al. (2009)	MSCs	Allogeneic	Transfection (VEGF siRNA)	2×10^6/kg body weight	Intracarotid artery	S
Tsuda et al., 2010	FM-MSCs	Allogeneic	Transgenic mouse (GFP)	5×10^5	Intravenous	I, F, D
Tsuda et al., 2014	FM-MSCs	Allogeneic	Transgenic mouse (GFP)	5×10^4	Intravenous	I, F, D
Uchida et al., 2017	BM-MSCs derived Muse cells	Xenogeneic (human)	Lentiviral transduction (GFP)	2×10^4	Intravenous	D, T
van Koppen et al., 2012	BMCs	Allogeneic	Transgenic rat (EGFP)	5×10^7	Intrarenal artery	I, A, D
van Koppen et al., 2015	BMSCs	Allogeneic	Transgenic rat (GFP)	5×10^7	Renal artery	I, A
Wang et al., 2015	BMSCs	Allogeneic	Lentiviral transduction (RFP)	1×10^6	Intravenous	I, D
Wang, Zhang, Zhuo, Wu, Xu, et al., 2016	BMSCs	Allogeneic	Transfection (CXCR-4)	2×10^6	Intravenous	D
Wang, Zhang, Zhuo, Wu, Liu, et al., 2016	BMSCs	Allogeneic	Lentiviral transduction (RFP)	1×10^6	Intravenous	I, D
Wise et al., 2014	MSCs	Xenogeneic (human)	NR (EGFP, Luciferase)	1×10^6	Intravenous	F, D
Yamagishi et al., 2001	BMCs	Allogeneic	Adenoviral transduction (IL-1ra, glucocerebrosidase)	5×10^6	Intravenous	I, F
Yokote et al., 2017	MSCs	Allogeneic	Transgenic rat (Luciferase)	5×10^5	Intravenous	D
Yuan et al., 2017	MSCs	Allogeneic	Transfection (miR-223 specific inhibitor)	2×10^6	Intravenous	I, F, A
Yuzeng et al., 2014	MSCs	Allogeneic	Lentiviral transduction (Survivin, EGFP)	1×10^6	Intravenous	D
Zhen-Qiang et al., 2012	BM-MSCs	Allogeneic	Adenoviral transduction (hBMP7, GFP)	NR	Intrarenal artery	A, D
Zhu et al., 2017	ADMSCs	Allogeneic	Transgenic mouse (GFP)	NR	Intravenous	D
Zhuo et al., 2013	MSCs	Allogeneic	Lentiviral transduction (Luciferase, RFP)	1×10^6, 2×10^6 and 5×10^6	Intravenous, intrarenal artery	D

I – Immune and Inflammation markers or related outcomes; F – Fibrosis related outcomes; A – Apoptosis; D – Biodistribution; T – Tumor and teratoma; S – Survival; 4E - Kidney-derived clonal cell line of MSC; ADMSCs - Adipose-derived mesenchymal stem cells; AF-MSCs - Amniotic fluid-derived mesenchymal stem cells; AFSCs – Amniotic fluid-derived stem cells; ASCs – Adipose stem cells; BFP – Blue fluorescent protein; BMDCs - Bone marrow-derived cells; BM-MSCs - Bone marrow-derived mesenchymal stem cells; BM-MSCs-derived Muse cells – Bone marrow mesenchymal stem cells-derived Multilineage-differentiating stress-enduring cells; COL4A3 – Collagen type IV alpha 3 chain; CTNS – Cystinosin; cUCB-MSCs – Canine umbilical cord blood-derived mesenchymal stem cells; CXCR4 – C-X-C chemokine receptor type 4; EC – Endothelial cells; EPCs - Endothelial progenitor cells; EPO – Erythropoietin; FM-MSCs – Fetal membrane-derived mesenchymal stem cells; GDNF – Glial cell-derived neurotrophic factor; GFP – Green fluorescent protein; GSTM2 - Glutathione S-transferase M2; HGF – Hepatocyte growth factor; HIF-1α – Hypoxia-inducible factor 1 alpha; HO-1 – Heme oxygenase; hPAP – Human placental alkaline phosphatase; hRPTEC - Human renal proximal tubular epithelial cells; HSPCs - Hematopoietic stem and progenitor cells; hucMSCs - Human-umbilical cord-derived mesenchymal stem cells; IGF-1 – Insulin-like growth factor 1; IL-4 – Interleukin-4; iPS – Induced pluripotent stem cells; Lcn2 – Lipocalin-2; MRPC/MKPCs - Mouse renal/kidney progenitor cells; MSCs - Mesenchymal Stem/Stromal Cells; Myh9 – Myosin-9; NGAL - Neutrophil gelatinase-associated lipocalin; NRK-52E - Rat epithelial kidney cell line; RFP – Red fluorescent protein; rKS56 - Proximal tubule S3 segment-derived renal progenitor-like cell line; RPCs – Renal progenitor cells; S1pr3 - Sphingosine-1-Phosphate Receptor 3; SAA1 – Serum amyloid A1; SIRT1/3 – Sirtuin 1/3; SRCs - Selected renal cells; TERT – Telomerase reverse transcriptase; TGF-β – Transforming growth factor beta; Tie-2 – Angiopoietin receptor; USCs – Urine-derived stem cells; VEGF – Vascular endothelial growth factor. * Renal tubule cells - Mix of cells derived from proximal, ascending, collecting and distal tubule. T* - Indirect evidence of tumorigenicity assessment (see Table 3).

3.4. Immunogenicity, cell rejection and tissue damage

Considering cell source and their potential immunogenic effect, all studies were evaluated for cell origin used for kidney disease treatment. The data obtained show that two studies used autologous cell transplantation, twelve used xenogeneic cells, eighty-two studies described the use of allogeneic cells, while one study did not report the origin of cells used. Reported parameters that were taken into account for possible assessment of inflammatory and immunogenic effects were predominantly cytokine expression and release, as well as inflammatory cell infiltration. In total, forty-seven studies were examined and showed at least one of the following outcomes: mRNA or protein levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 17 (IL-17), interleukin 10 (IL-10), interleukin 1 alpha (IL-1α), interleukin 1 beta (IL-1β), monocyte chemoattractant protein 1 (MCP-1), interferon gamma (IFN-γ), or infiltration of leukocytes and ED-1 positive cells. Furthermore, forty studies evaluated the presence of fibrotic markers such as mRNA or protein levels of TGF-β, α-SMA, PDGF, type I collagen and matrix metalloproteinases (MMP 2 and 9), or the extent of fibrotic tissue damage by trichrome periodic acid-Schiff or Sirius Red staining.

Similar findings were observed in case of apoptosis, which was considered as a possible indicator of cell rejection, as suggested previously (Cristobal et al., 2010; Krams et al., 1995). In total, thirty-two studies reported at least some of the apoptosis-related markers, such as caspase 3 expression, DNA fragmentation (TUNEL staining), Bax and Bcl2 expression. However, all of these studies were focused on reporting the protective effect on kidney tissue damage in terms of reduced number of apoptotic tubular cells, rather than possible adverse effects of cell therapy. As discussed in later sections, several studies evaluated biodistribution and specifically persistence of cells in kidneys, which could be suggestive of cell survival or rejection following administration.

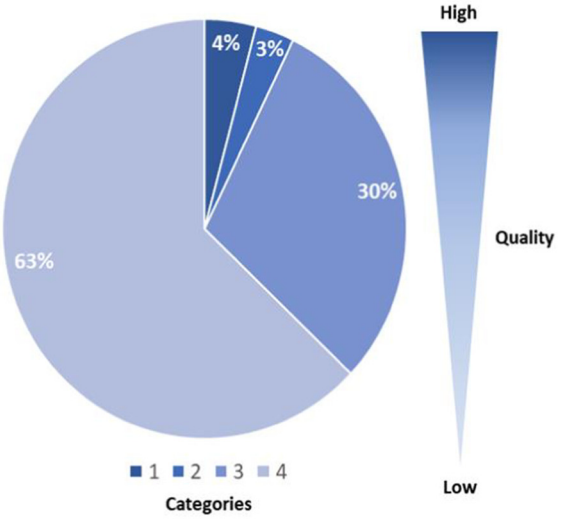
3.5. Tumorigenicity, oncogenicity and teratoma formation

Another important risk factor for cell-based therapies is the risk of tumorigenic and oncogenic effects, as well as teratoma formation. Overall, twelve studies assessed teratoma, tumor or other malignant occurrences (Table 3). The study by Caldas et al. (2017), in which iPS cells were used to treat subtotal nephrectomy-induced CKD in rats, showed that iPS cells induced formation of malignant tumors histologically resembling Wilms' tumor in 63% of the cases. Kinomura et al. (2008), who adopted Lac-Z transfected S3 segment-derived proximal tubule cells (rKS56), stated the tumorigenicity related findings indirectly (T*; Table 2) by referring to a previous study performed in nude mice, which suggested the absence of a tumor cell phenotype (Kitamura et al., 2005). However, according to the karyotype analysis, they found that rkS56 cells employed as cell therapy were nearly triploid regardless of LacZ transfection, and this could represent a risk of tumorigenesis (Giam & Rancati, 2015; Weaver & Cleveland, 2006). The study by Chen et al. (2011) assessed tumorigenic effect of human umbilical cord-derived mesenchymal stem cells (hucMSC) that were adenovirally-transduced with hepatocyte growth factor (HGF) and green fluorescent protein (GFP), in BALB/c nude mice instead of in the

A

Author and Year	Category
Bai *et al.* 2018	4
Bajwa *et al.* 2016	1
Bataille *et al.* 2016	3 -
Baulier *et al.* 2014	4
Bian *et al.* 2014	4
Bian *et al.* 2014(2)	2
Caldas *et al.* 2017	3 -
Chen *et al.* 2008	4
Chen *et al.* 2011	1
Chen *et al.* 2015	3 -
Du *et al.* 2016	3 -
Eliopoulos *et al.* 2011	4
Ezquer *et al.* 2015	4
Feng *et al.* 2016	3
Feng *et al.* 2018	4
Ferenbach *et al.* 2010	4
Franchi *et al.* 2014	3 -
Furuichi *et al.* 2012	4
Gao *et al.* 2012	4
Geng *et al.* 2014	3 -
Gheisari *et al.* 2012	4
Golle *et al.* 2017	4
Gregorini *et al.* 2016	3
Guiteras *et al.* 2017	3 -
Guo *et al.* 2006	3 -
Hagiwara *et al.* 2008	4
Han *et al.* 2013	4
Harrison *et al.* 2013	3
Huang *et al.* 2012	4
Huuskes *et al.* 2014	2
Imberti *et al.* 2007	4
Jia *et al.* 2016	4
Jiang *et al.* 2015	4
Kankuri *et al.* 2015	4
Katayama *et al.* 2008	4
Katsuoka *et al.* 2015	3
Kelley *et al.* 2013	3
Kelly *et al.* 2010	4
Kelly *et al.* 2012	4
Kelly *et al.* 2013	4
Kelly *et al.* 2015	4
Kinomura *et al.* 2008	3
Kluth *et al.* 2001	4
Kucic *et al.* 2008	1
LeBleu *et al.* 2009	4
Lee *et al.* 2012	3 -
Lee *et al.* 2017	3 -
Li *et al.* 2006	4
Li *et al.* 2006(2)	4
Li *et al.* 2012	3
Li *et al.* 2014	4
Li *et al.* 2015	3 -
Lin *et al.* 2003	4
Lira *et al.* 2017	4
Liu *et al.* 2011	4
Liu *et al.* 2013	4
Liu *et al.* 2015	4
Liu *et al.* 2016	4
Luo *et al.* 2014	4
Lv *et al.* 2014	4
Monteiro Carvalho Mori da Cunha *et al.* 2015	3 -
Monteiro Carvalho Mori da Cunha *et al.* 2017	4
Ornellas *et al.* 2017	3 -
Ozbek *et al.* 2015	4
Pacurari *et al.* 2013	3 -
Prodromidi *et al.* 2006	4
Rampino *et al.* 2011	3 -
Roudkenar *et al.* 2017	1
Ruan *et al.* 2013	4
Saito *et al.* 2012	3 -
Shuai *et al.* 2012	3
Si *et al.* 2015	4
Song *et al.* 2017	4
Sugimoto *et al.* 2006	4
Syres *et al.* 2009	4
Takahashi *et al.* 2013	4
Tang *et al.* 2018	4
Tian *et al.* 2017	4
Togel *et al.* 2008	2
Togel *et al.* 2009	3
Togel *et al.* 2009(2)	3
Tsuda *et al.* 2010	4
Tsuda *et al.* 2014	4
Uchida *et al.* 2017	3 -
van Koppen *et al.* 2012	3
van Koppen *et al.* 2015	4
Wang *et al.* 2015	4
Wang *et al.* 2016	4
Wang *et al.* 2016(2)	4
Wise *et al.* 2014	4
Yamagishi *et al.* 2001	4
Yokote *et al.* 2017	3 -
Yuan *et al.* 2017	4
Yuzeng *et al.* 2014	4
Zhen-Qiang *et al.* 2012	4
Zhu *et al.* 2017	4
Zhuo *et al.* 2013	4

B



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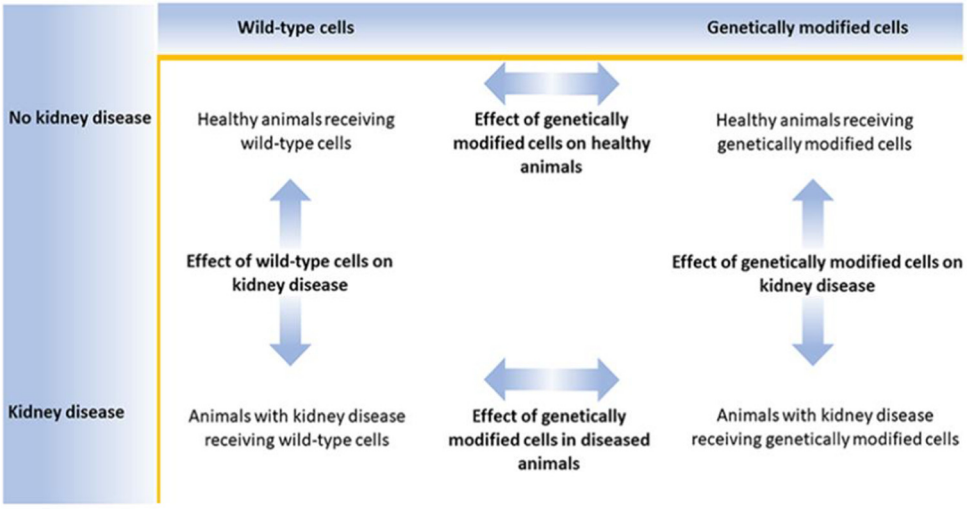

Fig. 2. Articles categorization based on suitability of study design and animal experimental set-up for determination of cell therapy risks and adverse events. Category 1 = Optimal study design (genetically manipulated and wild type cells administered in healthy control animals reporting adverse effects; Category 2 = Sub-optimal study design (genetically manipulated cells administered in healthy animals and monitoring for side effects; Category 3 = Sub-optimal study design with indirect evidence of adverse events (genetically manipulated cells administered only in disease animal models) and reporting adverse events; studies indicated as 3- represent those that did not report explicitly adverse events, even though outcomes

kidney disease animal model. Their results indicated the absence of tumor formation in nude mice during a three-months observation period. Moreover, several other studies reported that tumor generation following cell administration into kidney disease animal models was not observed. For instance, Togel, Cohen et al., (2009) showed that MSCs generated from human placental alkaline phosphatase (hPAP) transgenic F344 rats did not give rise to tumors in a Sprague Dawley rat AKI model, while Han et al. (2013) stated that there was no teratoma formation in kidneys of AKI C57BL/6 mice 6 weeks after injection of mouse adult renal progenitor cells derived from C57BL/6-GFP transgenic mice. Similarly, Li et al. (2012)) did not find any presence of teratoma in kidneys of mice with renal ischemic injury up to 6 months following injection of mouse hematopoietic stem and progenitor cells derived from transgenic CreKsp:R26R-EYFP mice and induced to differentiate into cells resembling renal cell phenotype. Moreover, endothelial progenitor cells transfected with telomerase reverse transcriptase did not cause any malignant changes in a chronic kidney disease setting (Shuai et al., 2012). Roudkenar et al. (2018) showed that lipocalin transfected MSCs were safe enough with no tumor formation observed even 2 months after administration of the cells in an AKI rat model, while the study by Uchida et al. (2017) showed the same result for GFP-lentivirally-transduced BM-MSCs-derived multilineage-differentiating stress-enduring (Muse) cells in a mouse model of glomerulosclerosis. Lee et al. (2012) and Harrison et al. (2013) showed in two different animal models of kidney disease that iPS cells reprogrammed with retroviral vectors encoding Oct-4, Sox2, Klf4 and EGFP, and hematopoietic stem and progenitor cells transduced with lentiviral vectors bearing CTNS and EGFP genes, respectively, did not induce tumor formation for the duration of the study. Finally, Li et al. (2015) stated that iPS-derived renal progenitor cells did not give rise to any neoplastic formations during 3 months follow-up in an AKI rat model. Overall, only one of the studies that monitored animals for tumor and teratoma formation showed the undesired effects, while the rest of them reported opposite outcome.

3.6. Biodistribution of administered cells

The in vivo fate and biodistribution of genetically modified cells are other important factors related to the safety of a cell-based therapy. The majority of the studies included in this systematic review (80; 82%) evaluated at least some biodistribution-related outcomes, such as trafficking, homing, engraftment, differentiation, survival, or persistence of cells after administration. Of these studies, forty-five (56%) focused only on cell engraftment and survival within kidneys, while thirty-five (44%) evaluated distribution in at least one or more additional organs, such as lung, liver, spleen, lymph nodes, stomach, intestine, muscle, brain, blood, bone marrow or eye. The specific cell type, organ or tissue distribution, cell persistence and outcome are shown in Table 4.

3.7. Contamination with microorganisms or adventitious agents and reactivation of latent viruses

Considering all included studies, only Takahashi et al. (2013) focused on safety issues related to pathogen contaminations. In particular, the study was performed to evaluate the effect of serum-containing medium on lifespan-extended renal proximal tubular epithelial cells function in the bioartificial tubule device (BTD). Namely, cells modified with siRNA for $p16^{INK4a}$ were cultured either in 0.5% serum-containing renal cell growth medium or serum-free RELAR® medium, based on HFDM-1 synthetic medium for human fibroblasts supplemented with various recombinant hormones and growth factors for renal cell culture. The results obtained showed that both cells cultured in serum-containing and in serum-free medium presented almost the same growth rate in terms of population doublings and performed equally in BTD with respect to leakage of creatinine and reabsorption of water, glucose and sodium. In addition, the two types of cells exhibited very similar performance in AKI goats during the 26 h extracorporeal circulation with BTD regarding plasma levels of liver enzymes, renal function parameters, glucose, and electrolytes, as well as mRNA levels of a number of cytokines following 8 h lipopolysaccharide (LPS) challenge of peripheral blood mononuclear cells (PBMC). This study suggests that it is possible to culture cells in pathogen-free conditions for cell therapy purposes. Regarding the possibility of reactivation of latent viruses, none of the studies included in this review addressed this issue.

3.8. Animal survival

Of the studies included, eighteen (19%) reported animal survival or mortality rate after cell administration (Table 2; outcome "S"). However, in all cases the main focus and intention was not to evaluate possible negative or detrimental effects of genetically modified cells and administration route, but to determine the beneficial effect of cell therapy on the overall survival in a kidney disease setting.

4. Discussion

Adequate reporting of measures to reduce bias and other key study quality indicators is crucial to assess risks of bias in primary studies and to determine the quality of a body of evidence. Our assessment indicates that animal studies in the renal regenerative medicine field are no exception to the insufficient reporting of preclinical animal studies in general. Since there is accumulating evidence that absence of measures to reduce bias can severely influence primary study results (Hirst et al., 2014), this is a matter of concern. Importantly, our review aimed to assess outcomes related to safety, but since 99% of the studies did not report a power calculation, it is impossible to assess whether any of the included studies were sufficiently powered to detect differences in these outcomes. This is a crucial point to take into consideration as it might lead to misinterpretation of the results as well as ethical issues with using inappropriate numbers of animals. For that reason, we strongly suggest to always perform a sample size calculation in animal studies by using either the power analysis, similarly to the methods employed for calculation of sample size in clinical studies, or the method called "resource equation" when several outcomes are measured and complex statistical analyses are needed (Charan & Kantharia, 2013; Faul, Erdfelder, Lang, & Buchner, 2007; Festing & Altman, 2002). Furthermore, since none of the included studies referred to a prospectively registered study protocol, risk of bias due to selective outcome reporting could not be assessed. Akin to clinical trials, where prospective registration of study protocols is the norm, prospective registration of animal studies can provide vital information to reviewers, readers and meta-researchers on e.g. the study hypothesis, endpoints and sample size calculation, and measures to reduce bias (Jansen of Lorkeers, Doevendans, & Chamuleau, 2014; Kimmelman & Anderson, 2012; Ritskes-Hoitinga & Wever, 2018). We therefore recommend prospective registration on e.g. preclinicaltrials.eu or Open Science Framework, and make a plea for the use of reporting guidelines such as ARRIVE and GSPC (Hooijmans, Leenaars, & Ritskes-Hoitinga, 2010; Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). Of note, these do not specify how detailed the reporting of measures to reduce bias should be. The guidance notes provided with SYRCLE's risk of bias tool (Hooijmans et al., 2014) offers many examples of how to report

relevant to side effects were present; Category 4 = Inappropriate study design (genetically manipulated cells administered in disease animal models not reporting adverse events or relative outcome measurements). A) List of included studies categorized as mentioned; B) Percentages of studies divided over 4 categories. C) Representation of an ideal study design in which both wild-type and genetically modified cells are used in healthy control animals and in animals with kidney disease. Such a study design would allow to determine both the efficacy and safety of a given type of genetically modified cells in certain model of renal disease.

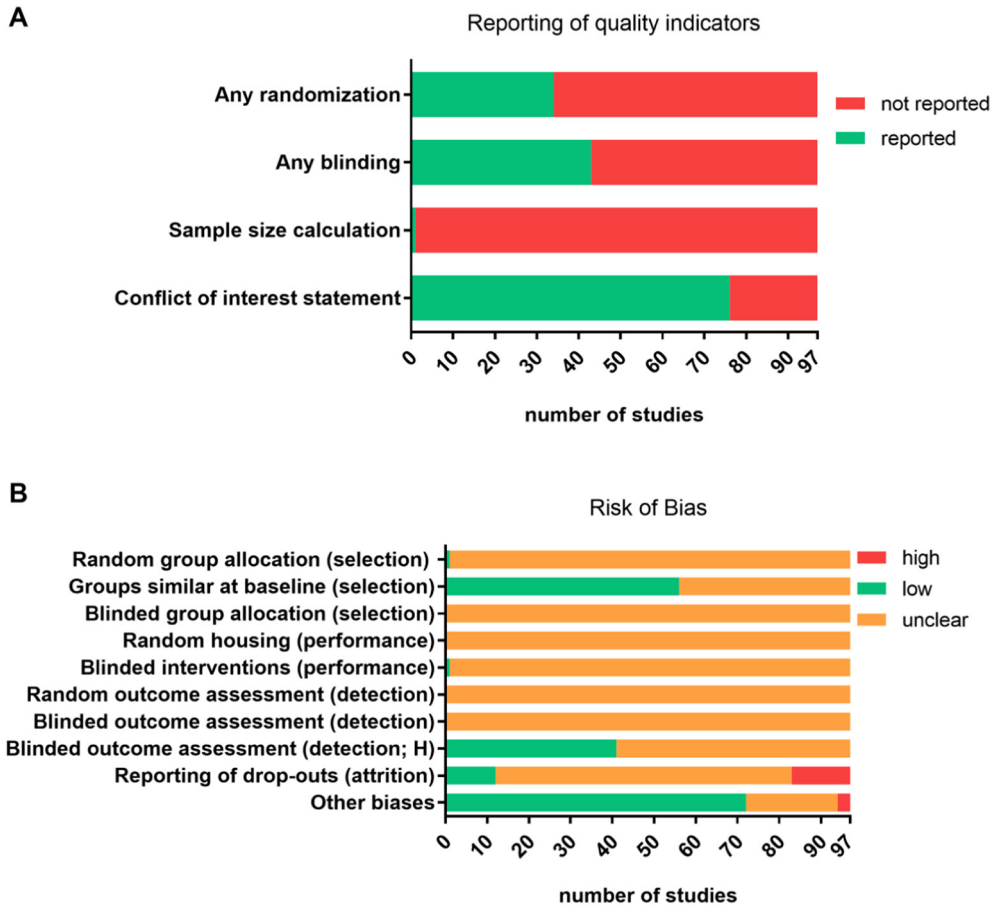

Fig. 3. Reporting quality and risk of bias assessment. A) Number of studies reporting measures to reduce bias and key quality indicators. B) Risk of bias in included studies regarding selection, performance, detection, attrition and other types of bias. H: blinding of the outcome measure by histology only.

measures to reduce various forms of bias in various stages of an animal experiment.

When considering immunogenicity-related risk factors, it could be expected that allogeneic and xenogeneic cells undergo rejection, thus compromising cell therapeutic activity, or even elicit undesired inflammatory and/or immune responses. This is especially related to the expression of highly polymorphic major histocompatibility complex antigens (MHC), as extensively studied and demonstrated in the solid organ transplantation field (Ingulli, 2010). Besides cell origin, which was reported by all studies included, several other parameters that might influence immunogenicity should be considered, including the administration site, the need for multiple exposures or administrations, the effect of disease on the immune system, as well as the maturation status of (stem) cells (Sharpe, Morton, & Rossi, 2012). It has been reported that some cells, such as embryonic stem cells (ESCs), although immunologically immature because they lack MHC class II molecules expression are not completely resistant to immune rejection (Drukker, 2008; Drukker et al., 2006; Li et al., 2004; Liu, Li, Fu, & Xu, 2017). In addition, most parameters related to immune and inflammatory responses reported by studies included in this review were obtained as an indication of efficacy of cell therapy and attenuation of kidney disease with beneficial anti-inflammatory effects, rather than as a safety evaluation of the cell therapy. Moreover, none of the studies examined systematically the expression of immune related antigens such as MHC Class I and II molecules or other co-stimulatory factors involved in immune system activation. From the observed data, it is not possible to infer any relevant conclusion concerning inflammatory and immune responses induced by specific cell types in kidney disease animal models, considering that there were no healthy or sham-operated animals treated with genetically modified cells and that the population of included studies was highly heterogeneous, especially in terms of animal species and renal disease models used, as well as specific cell type and their mode of application. In addition, in many cases mRNA or protein levels of pro-inflammatory mediators were assessed in peripheral blood or in kidney tissue lysates without discriminating the origin and localization of the inflammatory response within kidneys (glomerular or tubular injures), further hampering the interpretation of possible adverse effects.

Considering that fibrosis is a good indicator of tissue damage it could represent a valid marker for inflammatory response related safety aspects as well. However, fibrotic markers reported by included studies, similarly to inflammatory markers, were almost exclusively addressed for therapeutic goals of cell therapy and to evaluate the impact on renal fibrosis progression.

Furthermore, none of the included studies that reported any of the apoptosis-related markers had the optimal study design, nor determined the apoptosis status of administered cells, offering inconclusive results regarding cell persistence.

Tumorigenic and teratogenic effects of cell therapy are mostly due to the differentiation status of cells, or genetic manipulation and continuous cell culture that can lead to genetic aberrations and genomic alterations associated with cancer and tumor development (Laurent et al., 2011; Mayshar et al., 2010; Zhang et al., 2006). Even though almost all studies included in this review that monitored for tumor and teratoma formation reported promising findings and the absence of tumorigenic effects, one study showed that iPS cells were able to induce nephroblastoma formation in a CKD animal model (Caldas et al., 2017). This clearly indicates that genetically manipulated cells and especially stem cells could have negative consequences in cell-based therapeutic approaches. Therefore, when using genetically modified

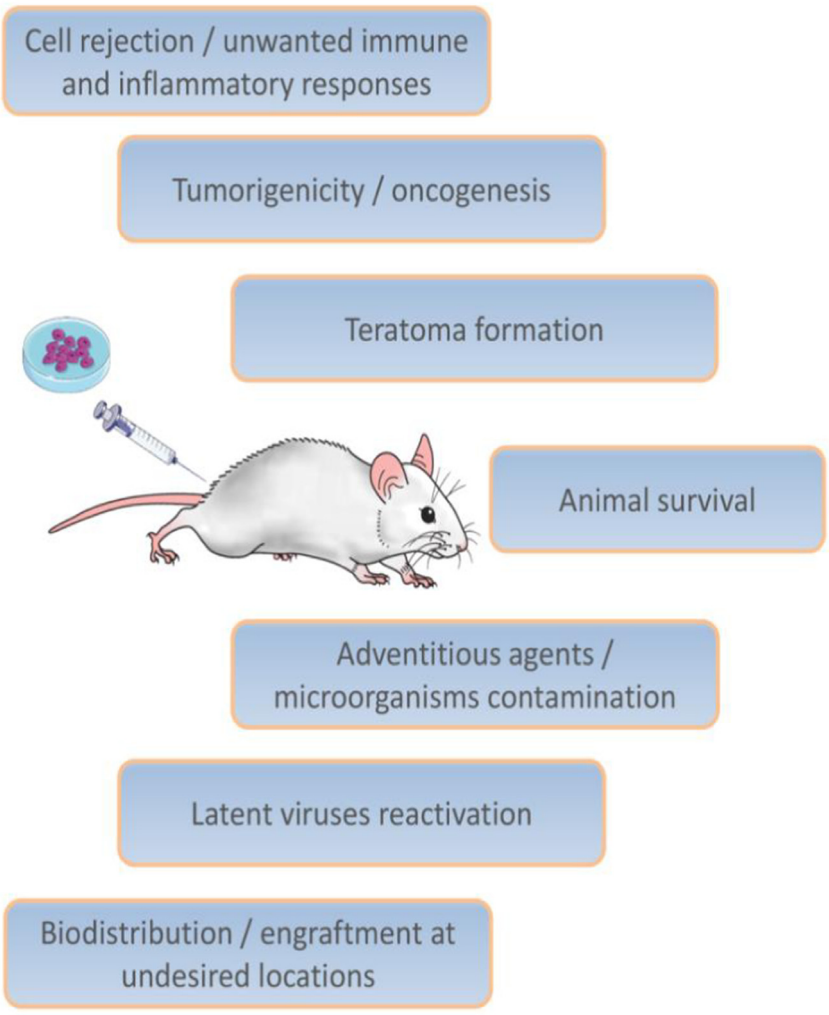

Fig. 4. Possible risk factors and adverse events related to cell administration for therapeutic purposes.

cells, it is essential to evaluate genetic stability of the cells as this can be significantly compromised. Not only genetic modifications, such as transfection and transduction, but also the use of cells derived from transgenic donors and the regular expansion and culture of cells, can be associated with the introduction of chromosomal aberrations and, thus, increase the risk of tumorigenicity in recipients (Rebuzzini, Zuccotti, Redi, & Garagna, 2015; Solomon, Borrow, & Goddard, 1991). Unfortunately, almost none of the studies included contained a karyotype analysis or other assessment of genetic stability, despite dealing with genetically modified cells or cells derived from transgenic animals. However, it should be recognized that we excluded studies using immunodeficient animals, such as severe combined immunodeficient (SCID)

Table 3
Tumor and teratoma formation assessment.

Author and Year	Species	Cell type	Tumor/teratoma formation outcome
Caldas et al., 2017	Rat	iPS	Wilm's tumor (nephroblastoma) formation detected during 2 months follow-up
Chen et al., 2011	Rat	hucMSCs	No tumor formation observed in nude mice during 3 months follow-up
Han et al., 2013	Mouse	MRPC	No teratoma formation observed during 6 weeks follow-up
Harrison et al., 2013	Mouse	HSPCs	No tumor formation observed during 12 months follow-up
Kinomura et al., 2008*	Rat	rKS56	Kitamura et al., 2005: No tumor formation observed in nude mice during 13 months follow-up
Lee et al., 2012	Rat	iPS	No tumor formation observed during 6 months follow-up
Li et al., 2012	Mouse	HSPCs (Lin-)	No teratoma formation observed during 6 months follow-up
Li et al., 2015	Rat	iPS-derived RPCs	No tumor formation observed during 3 months follow-up
Roudkenar et al., 2017	Rat	MSCs	No tumor formation observed during 2 months follow-up
Shuai et al., 2012	Rat	EPCs	No malignant changes observed during 3 months follow-up
Togel, Cohen, et al., 2009	Rat	MSCs	No tumor formation observed during 1 month follow-up
Uchida et al., 2017	Mouse	BM-MSCs derived Muse cells	No tumor formation observed during 7 weeks follow-up (tumorigenesis study is performed in FSGS-SCID mice)

BM-MSCs-derived Muse cells – Bone marrow mesenchymal stem cells-derived Multilineage-differentiating stress-enduring cells; EPCs - Endothelial progenitor cells; HSPCs - Hematopoietic stem and progenitor cells; hucMSCs - Human-umbilical cord-derived mesenchymal stem cells; iPS – Induced pluripotent stem cells; MRPC - Mouse renal progenitor cells; MSCs - Mesenchymal stem/stromal cells; rKS56 - Proximal tubule S3 segment-derived renal progenitor-like cell line; RPCs – Renal progenitor cells. * Indirect evidence of tumorigenicity.

Table 4
Organ and tissue distribution of various cell types used in kidney disease animal models.

Author and Year	Cell Type	Organ or Tissue Distribution	Cell Tracking Method	Outcomes
Bai et al., 2018	MSCs	Spleen	Fluorescence (RFP)	Cells present in spleen at 72 h after the administration.
Bajwa et al., 2016	BMDCs	Kidneys, lungs, spleen, liver, lymph nodes	Fluorescence (VT-680, intracellular staining; PKH26, cell membrane labeling)	Cells were detected mainly in the spleen up to 72 h post-injection, while no detection was observed in lungs, liver, kidneys or lymph nodes.
Bataille et al., 2016	BMDCs	Blood, bone marrow, kidney	Fluorescence (GFP)	At day 10 cells were present in blood, bone marrow and kidney tissue.
Baulier et al., 2014	AF-MSCs	Kidney, Lung, Spleen, Liver	Fluorescence (GFP)	Cells present in kidney 24 h after transplantation. Inconsistent presence of cells in kidney 3 months after administration. Cells were undetectable in lungs, spleen and liver both after 24 h and 3 months.
Bian, Zhou, et al., 2014	MSCs	Kidney	Fluorescence (GFP)	At day 28 cells were present in injured kidneys (tubular epithelium, interstitial space, glomeruli and peritubular capillary plexus).
Bian, Zhang, et al. (2014)	MSCs	Kidney	Fluorescence (GFP)	4 weeks after the administration cells were detected in injured kidneys (glomeruli, tubular epithelia, peritubular capillary plexus and interstitial space).
Caldas et al., 2017	iPS	Kidney	PCR (SRY male gene)	At day 60 cells were present in only in kidneys of animals with Wilms' tumor.
Chen et al., 2008	4E cell line	Kidney	Fluorescence (CM-Dil, α-SMA)	At 30 days after administration cells were present in injured kidney. Also, at day 30 cells were positive for α-SMA and localized along the peritubular capillary area indicating endothelial trans-differentiation.
Chen et al., 2011	hucMSCs	Kidney	Optical living body imaging	Cells were detectable in kidney 72 h after administration.
Chen et al., 2015	MKPCs	Kidney, lung	Fluorescence (GFP)	Cells were present in kidneys up to 7 days post-injection and almost non-detectable by the day 28. In the lungs, cells were detected 4 to 16 h post-injection and virtually not present at 9 weeks.
Du et al., 2016	Macrophages	Kidney, spleen, lung, liver	Fluorescence (EGFP)	Cells were mostly present in the lung and spleen 24 h post-injection and to a lower extent in the kidneys and liver. At day 7 after administration cells were almost not present in any tissue.
Eliopoulos et al., 2011	MSCs	Kidney	PCR (SRY male gene)	Cells were present after several days (up to 14 days).
Ezquer et al., 2015	MSCs	Kidney	Fluorescence (GFP)	Few cells present in kidneys at weeks 2 and 8 after administration.
Feng et al., 2016	ADSCs	Kidney	BLI (D-luciferase), Fluorescence (GFP)	Cells were present in kidneys up to 14 days after administration.
Ferenbach et al., 2010	Macrophages	Kidney, Liver, Spleen, Lung	Fluorescence (PKH26, cell membrane labeling)	24 h after injection high cell densities were identified in the spleen and liver, low cell densities were present in kidney and lung.
Franchi et al., 2014	MSCs	Whole body	BLI (D-luciferin) and Fluorescence (CM-Dil)	24 h after transplantation cells detected only in kidney. At day 3 after the administration cells localized mostly in glomeruli and persisted there for up to 4 weeks, with some cells expressing the endothelial marker vWF.
Furuichi et al., 2012	ADMSCs	Kidney, Lung, Brain, Spleen, Liver, Blood	Flow cytometry (GFP), IHC (anti-GFP)	After injection cells were mainly present in blood and lungs. Cells were present in all organs at day 4 post-injection, with decreased number in lungs. By day 7 after administration cells were almost disappear in all organs.
Gao et al., 2012	ADMSCs	Kidney	BLI (D-luciferase)	Cells highly present at day 1. Lower number of cells found at day 14. At day 21 cells were undetectable.
Geng et al., 2014	MSCs	Muscle, lung, kidney	Fluorescence (RFP)	24 h after administration cells were detected in the lung and gastrocnemius muscle, but not in injured kidneys.
Gheisari et al., 2012	MSCs	Kidney, Lung	Flow cytometry (CellTracker Green), Fluorescence (GFP)	36 h post-injection cells were present only in lungs but not in kidneys.
Golle et al., 2017	BMDCs	Kidney, heart	Fluorescence (GFP)	Cells were not detected neither in the heart nor in kidney tissue 2 weeks after administration.
Gregorini et al., 2016	MSCs	Kidney	Fluorescence (GFP)	At day 1 post-injection, few cells were detected kidneys mostly located in glomeruli, tubules and interstitium.
Guiteras et al., 2017	RAW 264.7 macrophages (cell line)	Whole body (liver, spleen, kidney, lung, heart, bowel, bladder)	Fluorescence (VT-680, intracellular staining)	At 48 h after administration cells were predominantly present in liver and to a lower extent in kidneys and spleen.
Guo et al., 2006	BMCs	Kidney	Fluorescence (GFP)	At day 1 after administration, cells were detected in injured kidneys.
Hagiwara et al., 2008	MSCs	Kidney	IHC (anti-human kallikrein)	Cells present in kidneys 48 h after administration.
Han et al., 2013	MRPC	Kidney	IHC (anti-GFP)	Cells present in kidney after 2, 4 and 7 days after administration.
Harrison et al., 2013	HSPCs	Whole body	Fluorescence (EGFP), BLI (D-luciferin)	Up to 9 months after administration cells were found present in spleen, liver (trans-differentiated into Kupffer cells), brain (trans-differentiated into microglial cells), kidney (trans-differentiated into inflammatory dendritic cells). Cells were still present 1 year after administration (shown by luciferase expression).
Huang et al., 2012	BM-MSCs	Kidney	Fluorescence (GFP)	Cells were detectable in kidney 72 h after administration.
Huuskes et al., 2014	BM-MSCs	Whole body	BLI (D-luciferase), Fluorescence (EGFP), PCR (EGFP)	Cells were present in injured kidneys 36 h post-injection.
Imberti et al., 2007	MSCs	Kidney	Fluorescence (PKH26, cell membrane labeling)	At day 4 post-injection cells were predominantly present in peritubular areas in kidneys.
Jia et al., 2016	BM-MSCs	Kidney	Fluorescence (GFP)	Cells were detectable in kidneys 8 weeks after administration.
Katsuoka et al., 2015	MSCs	Kidney, heart, brain, lung	BLI (D-luciferase)	Cells delivered intra-arterially were highly present in kidneys and lower body at day 1 post-injection. In case of the tail vein administration, majority of the cells were detected in the lungs 1 day after administration.
Kelley et al., 2013	Selected renal cells (SRCs)	Kidney	MRI (SPIO), Fluorescence (Rhodamine), NMR	Cells were present in kidneys at 24 h post-injection. Diminished yet significant presence 7 days after administration.

Table 4 (*continued*)

Author and Year	Cell Type	Organ or Tissue Distribution	Cell Tracking Method	Outcomes
			spectroscopy (CS-DM-Red (19F))	
Kelly et al., 2010	NRK-52E	Kidney, Spleen, Lung	Fluorescence (GFP)	At day 7 after administration cells were more abundant in kidney compared to lung and spleen.
Kelly et al., 2012	Renal tubular cells	Kidney, Lung, Spleen, Liver	Fluorescence (GFP), FISH (Y chromosome)	Cells present in kidneys 7 and 21 days post-injection. Cells were rarely detected in lungs, spleen and liver.
Kelly et al., 2013	Kidney tubular cells*	Kidneys, Lung, Liver, Spleen	FISH (Y chromosome), PCR (SRY male gene, SAA), Fluorescence (GFP)	Cells present in kidneys 14 weeks after the administration. At 14 weeks post-injection, only few cells were detected in lung, liver and spleen.
Kelly et al., 2015	Primary renal tubular cells	Kidneys, lungs, spleen, liver	Fluorescence (GFP), FISH (Y chromosome), PCR (SRY male gene, SAA)	Rare cells were detected in lungs, spleen or liver, but persistent detection of cells was found in injured kidneys.
Kinomura et al., 2008	rKS56	Kidney	IHC (Bluo-gal)	Following intra-arterial injection cells could not be detected in kidney. Upon subcapsular cell implantation cells were observed in the subcapsule and corticomedullary junction area at day 9 after transplantation.
Kluth et al., 2001	NR8383 (rat alveolar macrophage cell line)	Kidney	Fluorescence (PKH26, cell membrane labeling)	24 h after administration cells were predominantly detected in glomeruli.
Lee et al., 2012	iPS	Kidney, Liver, Spleen, Stomach, Intestine, Muscle, Lung, Brain	IHC (anti-GFP)	48 h after administration engraftment of cells was highest in the kidneys, in the peritubular area, followed by the spleen and the liver.
Lee et al., 2017	cUCB-MSCs	Kidney	Fluorescence (GFP)	28 days after administration, cells were mostly detected in renal cortex.
Li, Morioka, et al., 2006	BMDCs	Kidney	Fluorescence (EGFP)	At day 84 after administration cells were still present in kidneys (glomeruli).
Li, Deane, et al. (2006)	BMDCs	Kidney	Fluorescence (EGFP, CD31, vWF)	At days 7, 14 and 28 after injection, cells were present in kidneys and co-localizing with CD31 and vWF indicating trans-differentiation in endothelial cells.
Li et al., 2012	HSPCs (Lin-)	Kidney, Lung, Liver, Spleen, Bone marrow, Blood	FISH (Y chromosome)	At days 7 and 28 post-injection only few cells were present in kidneys. Higher number of cells detected in spleen, liver, lungs, bone marrow and blood at day 1 post-injection. At day 3 cell number was decreased in all organs.
Li et al., 2014	MSCs	Kidney	Fluorescence (GFP)	At 72 h post-injection cells were detected in kidneys.
Li et al., 2015	iPS-derived RPCs	Kidney	IHC (anti-GFP)	At week 2 post-injection cells were present in injured kidneys forming tubular structures.
Lin et al., 2003	HSPCs (Lin-)	Kidney	FISH (Y chromosome), PCR (SRY male gene), IHC (X-Gal)	4 weeks after transplantation cells were present in kidneys. Cells presence was confirmed in renal tubules by IHC.
Lira et al., 2017	MSCs	Kidney	Fluorescence (EGFP)	At 2 weeks after administration cells were still present in both renal cortex and medulla.
Liu et al., 2011	MSCs	Kidney, Brain, Liver	ISH (Y chromosome)	At 7 days after administration cells were detected in kidney (outer medulla). The number of cells decreased by day 14 post-administration. No cells were found in brain and liver.
Liu et al., 2013	BM-MSCs	Kidney	Fluorescence (BrdU, DNA labeling)	At day 7 after transplantation cells were present in kidney.
Liu et al., 2016	ADMSCs	Kidney	Fluorescence (PKH26, cell membrane labeling)	Cells were detected in kidneys at day 28 post-injection.
Luo et al., 2014	BM-MSCs	Kidney, Lung, Spleen, peritoenal lymph nodes	Fluorescence (RFP)	24 h after administration cells were found in the spleen, lymph nodes and the highest amount in lungs, but not in kidneys.
Lv et al., 2014	MSCs	Kidney	IHC (anti-GFP)	Small number of cells present around glomeruli and near vessels 24 h after transplantation. Only few cells present in glomeruli at 8 weeks.
Monteiro Carvalho Mori da Cunha et al., 2015	hAFSCs	Kidney, lungs, heart, spleen, liver	IHC (X-Gal)	Cells were detectable in kidneys and spleen 24 h post injection. At later time points (48 h and 2 months) no cells were present in the kidneys.
Ornellas et al. 2017	BMMCs	Kidney	Fluorescence (GFP)	Cells present in injured kidneys up to 24 h post-injection, after which cells were rarely detected.
Ozbek et al., 2015	MSCs	Kidney	Fluorescence (GFP, anti-VEGF)	At week 2 post-injection cells were detected in renal tubules and interstitium.
Pacurari et al., 2013	EC	Kidney, Liver, Lung, Heart, Spleen	Fluorescence (GFP, anti-CD31), IHC (vWF)	At day 7 after administration small number of cells was detected in kidney and spleen, while no cells were found in liver, lung and heart.
Prodromidi et al., 2006	BMDCs	Kidney	ISH (Y chromosome)	Cells were detected in kidneys (glomeruli and interstitium) after transplantation.
Rampino et al., 2011	MSCs	Kidney, Lung, Spleen	IHC (anti-GFP)	At 24 h after injection, cells were present in kidney (tubules, interstitium and glomeruli). At the same time, cells were occasionally present in lung and spleen. Cells also persisted in kidneys after 14 days (glomeruli).
Roudkenar et al., 2017	MSCs	Kidney	PCR (SRY male gene)	Cells were detectable in kidney tissue up to 12 days post-injection
Ruan et al., 2013	Fibroblasts (induced)	Kidney	Fluorescence (GFP)	At 8 weeks after transplantation cells were abundant in renal tubules, glomerular capillary loop and small arteries.
Si et al., 2015	MSCs	Kidney	Fluorescence (EGFP)	At day 3 cells were present in injured kidneys.
Song et al., 2017	ADSCs	Kidney	Fluorescence (GFP)	Few cells were present in the kidneys 24 h after administration.
Sugimoto et al., 2006	BMCs	Kidney	ISH (Y chromosome)	Cells were detectable in kidneys at week 13 following administration.

(*continued on next page*)

Table 4 (*continued*)

Author and Year	Cell Type	Organ or Tissue Distribution	Cell Tracking Method	Outcomes
Syres et al., 2009	BMDCs	Kidney, Eye, Brain, Muscle, Liver, Spleen, Heart	BLI (D-luciferin), Fluorescence (GFP), PCR (Ctns gene),	Cells were present at 2 and 4 months after injection. The number of cells increased over time in all organs.
Tian et al., 2017	USCs	Kidney	Fluorescence (GFP)	At day 7 post-injection cells were detected in kidneys (tubular epithelial cells).
Togel et al., 2008	MSCs	Whole body	BLI (D-luciferin), PCR (luciferase gene expression)	10–15 min after injection cells were located mostly in kidneys in AKI animals, while in healthy animals they show whole body distribution, especially in lungs. At 24 h post-injection cells are still present in kidneys and lungs of AKI animals. In liver no cells were detected. 7 days after injection cells were absent in lungs, kidneys, liver and spleen.
Tsuda et al., 2010	FM-MSCs	Kidney, Liver, Lung, Spleen	IHC (anti-GFP)	24 h after injection, cells were found in kidneys (glomeruli, proximal tubule, interstitial area), but also in lung, liver and spleen, with highest concentration in lungs, followed by liver, spleen and kidneys. At 7 days after injection, cells were not present in kidneys anymore, but still present in lung, liver and spleen.
Tsuda et al., 2014	FM-MSCs	Kidney, Lung, Spleen, Liver	IHC (anti-GFP)	24 h after administration cells were more abundant in lungs, but also present in liver, spleen and kidneys.
Uchida et al., 2017	BM-MSCs derived Muse cells	Lung, spleen, kidney, brain, liver, heart, muscle	Fluorescence (GFP)	At week 2 post-injection cells were present in injured kidneys (cortex and medulla) and to a lower extent in spleen and lungs. At week 7 post-injection cells were detectable only in the kidney cortex.
van Koppen et al., 2012	BMCs	Kidney	Fluorescence (EGFP)	Cells were present in kidneys at weeks 6 and 14 after administration.
Wang et al., 2015	BMSCs	Kidney	Fluorescence (RFP), PCR (RFP)	Cells were detected in injured kidneys up to 72 h after administration.
Wang, Zhang, Zhuo, Wu, Xu, et al., 2016	BMSCs	Kidney	Fluorescence (DAPI)	Cells were detected in injured kidneys up to 72 h after administration.
Wang, Zhang, Zhuo, Wu, Liu, et al., 2016	BMSCs	Kidney	Fluorescence (GFP), PCR (RFP)	Cells detectable in kidneys up to 72 h after administration.
Wise et al., 2014	MSCs	Kidney, Lung	BLI (D-luciferin)	1 h post-administration cells were present in lungs. Subsequently cells migrated to kidneys and remain persistent up to 3 days after injection. At day 7 post-administration cells were undetectable.
Yokote et al., 2017	MSCs	Whole body	BLI (D-luciferase)	Cells were initially detected only in the lungs, and disappeared within 2 days after administration.
Yuzeng et al., 2014	MSCs	Kidney	Fluorescence (GFP)	At day 7 after transplantation cells were detectable in kidney (survival of approximately 75%).
Zhen-Qiang et al., 2012	BM-MSCs	Kidney	Fluorescence (Hoechst33342 and anti-CK18)	At day 3 after transplantation cells were present in kidney renal tubules.
Zhu et al., 2017	ADMSCs	Kidney	Fluorescence (GFP)	Few cells were present in injured kidneys 5 days after administration.
Zhuo et al., 2013	MSCs	Whole body	BLI (D-luciferin)	Immediately after administration cells are localized in lungs. Cells disappeared completely at day 7 regardless the administration route.

4E - kidney-derived clonal cell line of MSC; ADMSCs - Adipose-derived mesenchymal stem cells; AF-MSCs - Amniotic fluid-derived mesenchymal stem cells; AFSCs – Amniotic fluid-derived stem cells; BLI – Bioluminescence imaging; BMDCs - Bone marrow-derived cells; BM-MSCs - Bone marrow-derived mesenchymal stem cells; BM-MSCs-derived Muse cells – Bone marrow mesenchymal stem cells-derived Multilineage-differentiating stress-enduring cells; CK-18 – Cytokeratin-18; cUCB-MSCs – Canine umbilical cord blood-derived mesenchymal stem cells; DAPI - 4′,6-diamidino-2-phenylindole; EC – Endothelial cells; (E)GFP – (Enhanced) Green fluorescent protein; (F)ISH – (Fluorescence) in situ hybridization; FM-MSCs – Fetal membrane-derived mesenchymal stem cells; HSPCs - Hematopoietic stem and progenitor cells; hucMSCs - Human-umbilical cord-derived mesenchymal stem cells; IHC – Immunohistochemistry; iPS – Induced pluripotent stem cells; MRI – Magnetic resonance imaging; MRPC/MKPCs - Mouse renal/kidney progenitor cells; MSCs - Mesenchymal Stem/Stromal Cells; NMR – Nuclear magnetic resonance; NRK-52E - Rat epithelial kidney cell line; RFP – Red fluorescent protein; rKS56 - Proximal tubule S3 segment-derived renal progenitor-like cell line; RPCs – Renal progenitor cells; SAA - Serum amyloid A; SPIO - Superparamagnetic iron oxide; SRCs - Selected renal cells; SRY – Sex-determining region Y; USCs – Urine-derived stem cells; vWF – Von Willebrand factor; α-SMA - α-Smooth muscle actin. * Renal tubule cells - Mix of cells derived from proximal, ascending, collecting and distal tubule.

mice, which are usually preferred for tumor xenograft evaluation. This was done because it was considered unjustified to combine data from immunodeficient and immunocompetent animals in one data synthesis. Moreover, a kidney disease model in immunodeficient animals does not precisely reflect the genuine pathophysiology.

One of the main biodistribution-related concerns is the undesired migration of cells to non-target organs and tissues, which could cause a risk of developing a local inflammatory response, or neoplasm formation. It has been reported previously that the number of cells reaching desired tissues and organs can be very low (as minimal as 1%) due to the fact that the cells remain trapped within the lungs due to their size and high abundance of surface adhesion molecules, especially in the case of an intravenous administration route (Ankrum & Karp, 2010; Schrepfer et al., 2007). Therefore, monitoring cell distribution and migration in the body is of crucial importance when assessing safety aspects of cell-based therapies.

Even though many studies included in this review reported biodistribution-related outcomes it is difficult to assess the overall biodistribution of genetically modified cells in kidney disease animal models due to large differences between studies in terms of cell type, administration route, number of cells, time point, and method implemented to trace cell fate after administration as well as kidney disease model applied. However, most studies included reported that cells could reach the kidney and survive for a variable period of time, ranging from 24 h up to one year. Also, all studies that evaluated distribution in organs other than the kidney mentioned cell accumulation in highly vascularized organs, such as spleen, liver and especially lungs. The study by Togel, Yang, Zhang, Hu, and Westenfelder (2008) focused exclusively on monitoring distribution of MSCs in a mouse model of AKI, using bioluminescence imaging (BLI). They showed that following intra-arterial injection in injured mice, cells tended to accumulate in areas corresponding to kidneys, while in healthy mice cells were distributed throughout the whole body, with eventual accumulation in the lungs. On the other hand, intravenous injection of cells led to a predominant and immediate cell accumulation in the lungs both in healthy and AKI mice. This implies that the concern of causing respiratory and

hemodynamic complications due to capillary clogging is paramount, especially in case of intravenous injections. Nonetheless, more than 50% of the biodistribution-relevant studies (Table 2) adopted the intravenous administration route for cell delivery. In addition, due to a low rate of retention and limited cell survival, large numbers of cells may be needed to achieve therapeutic effects, which would further increase the risk of pulmonary emboli. Besides this undesired effect of cell distribution on efficacy, engraftment of cells in non-target tissues might also be responsible for unwanted negative effects of cell therapies. For instance, different local environments could influence cell behavior and biological properties, thus potentially favoring harmful effects related to differentiation, especially if stem cells are used (Breitbach et al., 2007). However, none of the included studies reported differentiation issues. Nevertheless, in light of the evidence presented, biodistribution-related effects should not be underestimated, but carefully and extensively evaluated, especially in terms of mode and site of administration, cell number and type, as well as methodologies employed for cell tracking and detection.

The risk of transmission of pathogens, such as bacteria, viruses, fungi or prions, is another important, yet not sufficiently investigated safety concern of cells and tissue xenografts and allografts (Fishman, Greenwald, & Grossi, 2012; Greenwald, Kuehnert, & Fishman, 2012). This cannot be neglected, especially if the cells intended for use are of non-autologous origin and are genetically modified, cultured and expanded in vitro. Several studies have already described the undesired transmission of pathogens following cell transplantation (Kainer et al., 2004; Tugwell et al., 2005). Moreover, animal-derived products commonly used for the isolation, culture and propagation of cells, such as fetal calf or bovine serum, represent an additional risk of transfer of contaminants. In fact, due to its method of preparation, fetal bovine serum might be contaminated with mycoplasma, viruses, prions, or endotoxins, thus transmitting diseases. It also contains various biomolecules, such as non-human sialic acid, that could have xeno-immunogenic effects in hosts (Chieregato et al., 2011; Herberts et al., 2011; Jin, Xu, Champion, & Kruth, 2015; van der Valk et al., 2004; van der Valk et al., 2010). Therefore, in order to avoid ethical issues and controversial procedures involved in harvesting serum from bovine fetuses, and to ensure animal and pathogen-free conditions of cell culture for safer tissue engineering and cell therapy applications, alternatives for fetal bovine serum are highly desired.

Moreover, the possibility of reactivation of latent viruses, such as cytomegalovirus, herpes zoster or Epstein-Barr virus, with the production of infectious viral particles, is another crucial point to be addressed (European Medicines Agency, 2012), which unfortunately was not addressed in any of the included studies. However, we are aware that this aspect of safety is highly linked to the immunosuppressive therapy that might be required when allogeneic cells are applied, and that it can be more relevant in the clinical rather than the preclinical setting.

As mentioned, the experimental design of evaluated studies was often inappropriate for evaluation of the risks associated with cell administration. Even if the main goal of the studies was to assess a therapeutic effect, we believe that additional test groups could have been included to evaluate safety. Notably, the most frequently missing animal group was a control group (e.g. healthy or sham operated animals) in which the same cells were used as in the diseased group. In case of treatment with genetically modified cells, an additional control group of animals treated with wild type cells could also provide valuable information regarding the effect of cell therapy on overall animal well-being. For instance, considering animal survival, it would have been of great value if the studies reporting that outcome had the appropriate control groups, which could give a fair indication of cell therapy effect on survival, without confounding variables such as kidney disease itself. In that regard, most of the included studies that reported any results on animal survival, had a healthy or sham operated control group, depending on the kidney disease model, a vehicle (such as PBS) treated group, and a group treated with genetically modified cells for therapeutic purposes. On rare occasions a group of animals with kidney disease treated with wild type cells, as a control for the group injected with genetically modified cells was included as well. Nonetheless, in the absence of a healthy or sham operated control group treated with genetically modified cells alone, the potential adverse effects of cell therapy on animal survival were difficult to evaluate.

Besides, the studies included in this review are marked by high heterogeneity, especially in terms of the chosen cell type, cell number, route of administration, and, to some extent, cell source. In fact, cell numbers applied across all these studies were in the range of 10^3–10^8 (Table 2). This clearly indicates that the cell number required for optimal therapeutic results is not clear. Higher cell numbers might imply higher risk of developing certain side effects, such as lung obstruction (Schrepfer et al., 2007), but this also depends on the animal model and route of administration chosen, as reported previously (Togel et al., 2008). For that reason, a careful examination of cell biodistribution in animal experiments should be performed to determine the effectiveness and safety of a given cell type.

Another important issue is to ascertain the purity of a cell population that is intended for therapeutic use, even more so when cells underwent genetic modifications. With that in mind, the differentiation status of cells should be determined, by examining specific cell type markers and when possible cell specific functions, in order to avoid the undesired tumorigenic risks due to residual undifferentiated cells (Goldring et al., 2011). When poorly differentiated cells, or even stem cells, such as embryonic stem cells are used, the tumorigenic and teratogenic effects should be evaluated carefully (Blum & Benvenisty, 2008).

The determination of cell culture purity is also necessary to evaluate the genetic changes that can occur in culture over time, or are a consequence of various genetic manipulations, such as transfection or transduction used to enhance cell function. Karyotype analysis should suffice to determine any significant chromosomal aberrations that might render the cell product unreliable for use, either from the functional point of view or based on cancer risk.

Moreover, when possible, cells should be tested in animal studies at higher passage numbers, usually beyond the routine use, to ensure the safety related to tumorigenicity and immunogenicity, as already suggested ("WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks,", 2010). When using allogeneic or xenogeneic cells, it is extremely important to characterize cells for their immunogenic potential by assessing expression of immune related molecules and antigens, but also by evaluating cells persisting at the site of transplantation to check for cell survival, inflammatory cell infiltration, T cell activation, and cytokine and antibody levels, as indicators of graft rejection and immune system activation (Lee et al., 2014; Lefaucheur et al., 2013; Oliveira, Chagastelles, Sesterheim, & Pranke, 2017; Terasaki & Cai, 2005). In case of genetically modified cells, major attention should be given to the expression of transgenes, used either for improving cell function or for cell tracking, since the particular gene products can be immunogenic, as was shown for GFP (Ansari et al., 2016).

Finally, manufacturing processes of cells and cell-based therapies does not provide any viral removal or inactivation and sterilization. Considering that most cells are cultured and expanded in fetal bovine serum-containing growth media, continuous testing for microorganisms should be performed to maintain microbial safety of cell based therapeutic products (Herberts et al., 2011). In addition to ensuring microbial safety, use of serum-free culture media could help standardize the cell expansion and manufacturing procedures that are subject to serum batch-to-batch variations (3Rs-Centre, 2018; Jochems, van der Valk, Stafleu, & Baumans, 2002; van der Valk et al., 2004; van der Valk et al., 2010; Wessman & Levings, 1999).

Even though there are several official guidelines (European Medicines Agency, 2008, 2012; Food and Drug Administration, 2013; "WHO Recommendations for the evaluation of animal cell cultures as

substrates for the manufacture of biological medicinal products and for the characterization of cell banks,", 2010) for safety assessment of cell therapies and medicinal products based on cells and tissues, poor preclinical practice makes it difficult for regulatory agencies to establish new or improve currently existing guidelines and recommendations for safety evaluation. Overall, better design and execution of preclinical studies could drastically improve the safety evaluation of genetically modified cell therapy in kidney disease.

5. Conclusions

We identified ninety-seven studies describing the use and efficacy of genetically modified cells for the treatment of AKI and CKD in animals. However, only seven of these studies (7%) assessed the safety aspects of such therapies in a sufficient manner. Based on the current findings and observations it seems that most cell types employed for kidney disease treatment do not carry significant risk factors and side effects. However, given that most of the studies did not have the optimal design, with 63% designed poorly or inappropriately, and that they were highly heterogeneous with respect to animal species, disease model and cell therapy, it is rather challenging to get a general overview on safety aspects of cell-based therapies. Furthermore, reporting of measures to reduce bias and key study quality indicators was poor in nearly all studies, rendering all studies at unclear risk of bias, which decreases our confidence in the results. Hence, we encourage further research with well-designed preclinical studies according to the guidelines and recommendations, in order to better define adverse events potentially involved in kidney cell therapy. The most relevant safety-related outcomes are those regarding the purity, biodistribution and immunotoxic effects, as well as the tumorigenic potential related to genetic modifications, genomic instability and differentiation level of cells. Finally, we would like to extend the importance of well-designed and performed preclinical studies for cell-based therapies on other fields as well, such as heart, pulmonary, gastrointestinal, liver and other diseases and that it is worth sounding an alarm bell on the current style of scientific papers describing the use of animal models.

Conflict of interest statement

Authors declare no conflict of interest.

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Appendix A. Supplementary data

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