

Concise Reviews: Stem Cells and Kidney Regeneration: An Update

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ABSTRACT

Significant progress has been made to advance stem cell products as potential therapies for kidney diseases: various kinds of stem cells can restore renal function in preclinical models of acute and chronic kidney injury. Nonetheless this literature contains contradictory results, and for this reason, we focus this review on reasons for apparent discrepancies in the literature, because they contribute to difficulty in translating renal regenerative therapies. Differences in methodologies used to derive and culture stem cells, even those from the same source, in addition to the lack of standardized renal disease animal models (both acute and chronic), are important considerations underlying contradictory results in the literature. We propose that harmonized rigorous protocols for characterization, handling, and delivery of stem cells in vivo could significantly advance the field, and present details of some suggested approaches to foster translation in the field of renal regeneration. Our goal is to encourage coordination of methodologies (standardization) and long-lasting collaborations to improve protocols and models to lead to reproducible, interpretable, high-quality preclinical data. This approach will certainly increase our chance to 1 day offer stem cell therapeutic options for patients with all-too-common renal diseases. *STEM CELLS TRANSLATIONAL MEDICINE* 2019;8:82–92

SIGNIFICANCE STATEMENT

This review reports an update on the use of stem cells for renal regeneration. It is proposed that harmonized rigorous protocols for characterization, handling, and delivery of stem cells in vivo could significantly advance the field of translational renal therapies.

INTRODUCTION

Both preclinical reports and clinical trials of stem cells used for treatment kidney disease are increasing rapidly. Different types of stem cells, ranging from mesenchymal stem cells (MSCs) [1, 2], adipose stem cells (AdSCs) [3], amniotic fluid stem cells (AFSCs) [4, 5], renal progenitors (RPs) [6, 7], and so forth, can stimulate renal repair in vivo in models of acute and chronic kidney failure. These stem cells act in part as modulators of fibrotic/inflammatory pathways and stimulate endogenous regeneration and repair mechanisms. Nonetheless, translation of renal stem cell therapies appears to be somewhat stalled, prompting us to re-evaluate the literature with an eye toward methodological details.

We highlight a variety of stem cell approaches in preclinical development for acute kidney injury (AKI) and chronic kidney disease (CKD). Our goal is to provide examples of seemingly contradictory results and evaluate differences and similarities between studies. From this analysis, we provide our opinion for the most promising paths forward

for the use of stem cells in renal regeneration and to speed translation of these cells. The activation of endogenous renal cells for kidney regeneration, embryonic and pluripotent stem cells for the generation of renal organoids, and kidney-on-a-chip technologies are not included in this analysis, and readers are referred to relevant interesting reviews [8, 9]. Similarly, pluripotent stem cells used to create nephron organoids [10] and more complex kidney organoids [11, 12] are not discussed. We also do not address the myriad engineered adjuncts to stem cell therapies, or genetically manipulated stem cells or renal tissue engineering.

DIFFERENT TYPES OF STEM CELLS AND PRECLINICAL MODELS OF RENAL DAMAGE

In this section, we will highlight different stem cell types and animal models of AKI and CKD.

Several different populations of human and murine resident RPs, both adult and embryonic, have been studied for renal regeneration. In particular, human CD133⁺ cells (from infant

or adult kidneys) provide protection in murine models of acute tubular and glomerular damage [6, 13]. Administration of CD133⁺ human cells reportedly limited fibrosis after AKI and promoted erythropoietin production [13]. Similar cells can be obtained from urine of adult humans [14]. Human fetal kidney-derived NCAM⁺ progenitors administered into kidney parenchyma in a NOD/SCID mouse model of CKD created by nephrectomy of 5/6 of the kidney mass (5/6 Nx) engrafted, mediated improved creatinine clearance, and reduced fibrosis [15]. Interestingly, RPs co-expressing stromal and epithelial markers in urine of preterm neonates seem to be a readily available source for similar cells [16]. Many different types of progenitor cells of mouse/rat origin have been isolated using slow-cycling properties of stem cells or by stem cell marker expression and demonstrate beneficial effects in acute and chronic models of renal damage [17].

A number of studies examined resident populations of mesenchymal stromal cells (MSC) from glomeruli and renal interstitium. When compared with nonrenal MSC, the resident population has a more nephrogenic gene expression profile, suggesting propensity to transdifferentiate or differentiate into renal cells under appropriate cues. Indeed, they can differentiate not only into renal epithelial cells but also into erythropoietin-producing fibroblasts and juxtaglomerular cells containing renin granules [18]. Nestin⁺ renal MSC mediate rescue of AKI in mice, reducing serum creatinine and blood urea nitrogen (BUN) levels, and host apoptotic events. Paracrine protective effects of this population include vascular endothelial growth factor (VEGF) signaling, essential in maintaining the glomerular basement membrane [19]. These kidney-derived cells are unlikely to be developed for autologous use as the population is exhausted over the course of progression of CKD.

MSCs of nonrenal origin (bone marrow, adipose tissue, placenta, cord and blood) are the most studied cells in animal models of AKI and CKD. In AKI, either induced by toxic agents such as cisplatin or glycerol, by sepsis, or by ischemia-reperfusion (I-R), MSCs have shown protective and regenerative effects [20]. In CKD, accumulating experimental evidence indicates that MSC treatment can reduce fibrosis and renal dysfunction [21]. MSCs limited CKD progression in 5/6 Nx models [22] and ameliorated glomerular pathology in rodent models of diabetic nephropathy [23, 24]. A meta-analysis performed in 2015 showed that administration of bone marrow-derived MSCs [compared with induced-pluripotent stem cells (iPSCs) or organ-specific MSC] were most effective cell type in slowing development and progression of CKD [25]. Reduction of BUN and improved glomerulosclerosis and interstitial fibrosis pathologies were the most relevant changes observed after MSC therapy. After glomerular damage induced by adriamycin [26] MSCs can improve glomerular function. In addition to amelioration of renal morphological abnormalities and decreased proteinuria, pathological inflammation and microvessel rarefaction can also be improved by MSCs [27].

Therapeutic signals in preclinical models were also obtained using adipose-derived MSCs (AdMSCs). These cells have been tested in most models of AKI, and the results are encouraging [28, 29]. A stromal vascular fraction (SVF) can be isolated from adipose and directly administered without culture, making “same surgical procedure” possible for anticipated autologous approaches. (It is important to note that isolation of SVF, which requires centrifugation steps and usually enzymatic digestion

makes these cells “more than minimally manipulated” by FDA guidance.) SVF-mediated renoprotective effects are similar to those of cultured adipose MSCs in a rat model of I/R injury [30]. MSCs from cord blood show similar effects to bone marrow MSCs in models of both AKI and CKD [31].

MSCs from older renal failure patients can be used autologously, but their therapeutic efficacy may be blunted with age (and disease). The complex disease pathological milieu is known to affect MSC function such that MSCs from uremic or diabetic patients are likely to be less effective therapeutically [32, 33] compared to that of MSCs from healthy donors.

Endothelial progenitor cells (EPCs) from marrow or peripheral blood also have therapeutic potential in renal disease. EPCs mainly act by promoting neovasculation, via both paracrine signaling and in situ differentiation into mature endothelial cells. In pigs, EPCs ameliorated postischemic injury due to renal arterial stenosis [34]. However, when directly compared with MSCs, the renoprotective effect of EPCs was slightly inferior. In rat models of glomerulosclerosis, unfractionated bone marrow-derived cells (BMDCs, containing MSCs and precursors of EPCs) ameliorated disease by engrafting into the microvasculature of the glomerulus, suggesting that the potential of BMDCs to differentiate toward endothelial lineages confers rescue [35]. Similar to MSCs, BMDCs from diseased animals can shift toward a smooth muscle/myofibroblast progenitor cell lineage, reducing their efficacy. Maintaining the balance between SPCs and EPCs and mobilizing, recruiting, homing, and engrafting endothelial-committed cells are complex goals for optimizing EPC therapies [35]. Although regeneration of the vasculature of an entire organ continues to be challenging, EPCs are capable of preserving existing microvasculature [36]. The relative advantages of lineage-restricted EPCs versus MSCs with greater differentiation potential are likely to be kidney disease type-specific and are not yet defined.

We demonstrated that stem cells derived from amniotic fluid are renoprotective in animal models of glycerol-induced AKI, as well as CKD [4, 37]. AFSC seem to protect tubular and glomerular cells by stimulating endogenous repair mechanisms, thus reducing inflammation and fibrosis [38]. Other groups showed that these cells enhance recovery in 5/6 Nx models and cisplatin-induced toxicity [39].

iPSCs are another attractive (potentially patient specific) source of cells for treating kidney disease. Morigi's group reported that administration of human iPSCs, differentiated toward nephrogenic intermediate mesoderm and then metanephric mesenchyme, could engraft and promote recovery from cisplatin-induced AKI [40]. Similar beneficial results were obtained in a mouse renal I/R model. Human iPSC-derived RPs transplanted under the kidney capsule limited renal dysfunction and fibrosis development [41]. Less work has been performed with ESCs in the context of renal failure. Nevertheless, encapsulated mouse ESCs transplanted onto the omentum slowed progression of renal disease in a rat 5/6 Nx model [42].

Although not an exhaustive list of stem cell-based approaches to preclinical kidney disease, the aforementioned studies highlight diversity in the source, the characterization, the application, and the mechanism of action of stem cells; the diversity of species studied and disease models. Table 1 is a summary of types of stem cells used, route of delivery, and disease models and species for experimental renal disease therapies.

Table 1. Summary of stem cell therapy models for kidney disease

Model	Species	Cell	Route	References
Acute kidney injury				
I/R	Mouse	Glomerular progenitors and tubular progenitors	IV	[45]
I/R	Mouse	Mouse clonal renal MSC	IV	[18]
I/R	Mouse	Nestin ⁺ kidney MSC	IV	[19]
I/R	Sheep	Autologous BM-MSC	Renal artery	[48]
Kidney autotx (I/R)	Pig	Autologous AF-MSC	Renal artery	[4]
I/R	Rat	AdMSC	IV	[29]
I/R	Rat	hAdSVF, hAdMSC	Renal parenchyma	[30]
I/R	Mouse	hiPSC-RPs (OSR1 ⁺ SIX2 ⁺)	Renal capsule	[41]
I/R	Rat	c-kit ⁺ renal cells	IP, intraaortic	[101]
Ureter obstruction	Rat	rADSC	IV	[28]
Sepsis	Rat	Autologous AdMSC	IV	[102]
Glycerol	Mouse	hCD133 ⁺ RPs	IV	[13]
Glycerol	Mouse	hCD133 ⁺ CD24 ⁺ progenitors	IV	[55]
Glycerol	Mouse	hAFSC	Renal parenchyma	[38]
Glycerol	Mouse	hMSC-microvesicles	IV	[44]
Glycerol	Mouse	hBM-MSC	IV	[70]
Cisplatin	Mouse	mBM-MSC	IV	[20]
Cisplatin	Mouse	hUC-MSC	IV	[31]
Cisplatin	Mouse	hiPSC-RPs	IV	[40]
Cisplatin	Rat	hAF-MSC	IV	[39]
Cisplatin	Rat	hUC-MSC exosomes	Renal capsule	[62]
Cisplatin	Rat	Allo-rBM-MSC	IV	[67]
Cisplatin	Rat	hADSC hAFSC hCD133 ⁺ kidney cells	IV	[53]
Cisplatin	Macaque	Autologous BM-MSC	Intraarterial	[49]
Adriamycin	Mouse	EPC (in hydrogel)	SC in ear Renal capsule	[103]
Adriamycin	Rat	rBM-MSC	IV	[26]
Gentamycin	Dog	cUC-MSC	Renal parenchyma	[104]
Chronic kidney disease				
Renal artery stenosis	Pig	Pig MSC, EPC	Renal artery	[34]
Renal artery stenosis	Pig	Autologous EPC	Renal artery	[36]
RAS with metabolic syndrome	Pig	Autologous MSC-EV	Renal artery	[105]
5/6 Nx	Mouse	Human NCAM ⁺ RPs	Renal parenchyma	[15]
5/6 Nx	Rat	rBM-MSC	IV	[22, 27]
5/6 Nx	Rat	mESC	Gel-on renal remnant Gel-on omentum	[42]
5/6 Nx	Rat	rBM-MSC hiPSC	Renal parenchyma	[106]
Alport syndrome	Mouse	AFSC	Intracardiac	[5]
Alport syndrome	Mouse	AFSC-EV	Intracardiac	[43]
Lupus nephritis	Mouse	hESC-MSC	IV	[61]
Spontaneous CKD	Cat	Allo-AdMSC	IV	[50]
Diabetic nephropathy	Mouse	mAFSC	Renal parenchyma	[72]
Diabetic nephropathy	Mouse	hBM-MSC	Intracardiac	[23]
Diabetic nephropathy	Mouse	mBM-MSC	IV	[24]
Diabetic nephropathy	Rat	rBM-MSC	Renal artery	[107]

Examples highlighting the wide range of preclinical models and species studied in the context of stem cell therapies for kidney disease. Routes of delivery are constrained by animal model and change biodistribution, so are noted here. Abbreviations: I/R, ischemia reperfusion; autotx, autotransplantation; IP, intraperitoneal; RAS, renal artery stenosis; Nx, nephrectomy; MSC, mesenchymal stem cell; BM, bone marrow; AF, amniotic fluid; Ad, adipose; SVF, stromal vascular fraction; iPSC, induced pluripotent stem cell; ADSC, adipose-derived stem cell; UC, umbilical cord; EPC, endothelial progenitor cell; SC, stem cells; EV, extracellular vesicles; RPs, renal progenitor; AdMSC, adipose-derived MSC; AFSC, amniotic fluid stem cells Before a stem cell type; h, human; m, mouse; c, canine; r, rat; allo, allogeneic.

INTERPRETING REPORTS OF STEM CELL THERAPIES FOR KIDNEY DISEASE

It is generally accepted that the renoprotective activity of stem cells (both in acute and chronic renal disease models) is due to stem cell secretion of cytokines and other molecules that inhibit inflammation and fibrosis and promote endogenous repair processes including angiogenesis. Lately, several groups including ours demonstrated that a major mechanism of action of stem cells is via secretion of extracellular vesicles (EVs) [43–45]. When taken up by host renal cells, EVs can transfer genetic information that promotes regenerative processes. We also demonstrated that EVs can “trap” ligands on EV membrane surface receptors. For example, VEGF is trapped by surface VEGF receptors on AFSC-derived EVs, thus sequestering VEGF away from host glomerular cell receptors, which inhibits pathological VEGF signaling involved in perpetuating renal injury [43]. More details on the mechanisms of action of stem cells can be found in Refs. [46, 47].

A general bottleneck in the field is the apparent difficulty in reproducing rodent studies in larger animal models (cats, sheep). A careful look at these studies suggest that the sources of cells and the details of the animal models in part account for this problem, in addition to distinct renal physiology based on species. For instance, after ovine I/R injury, autologous MSCs did not provide therapeutic benefit [48], which would have been expected from similar rodent studies. Likewise, in the rhesus monkey, MSC administration ameliorated AKI induced by cisplatin to some extent but did not reverse established interstitial fibrosis [49]. Autologous AdMSCs have been the primary cell source used in feline preclinical models, but none of the studies have been able to replicate the efficacy reported in rodents [50, 51]. Differences between rodent and feline kidneys certainly explain some of these results, which are discouraging as feline CKD more closely resembles human pathology. Feline MSCs have been characterized [52] but not rigorously compared with rodent or human MSCs.

IMPORTANT VARIABLES IN THE PRECLINICAL RENAL REGENERATION LITERATURE

Stem Cell Source, Isolation, and Culture

Standardized protocols for the characterization, identity, culture, and differentiation of the many stem cell types used in renal regeneration research are not yet fully established. For example, Santeramo et al. raised the issue of specificity of RPs by showing a similar degree of renal regeneration induced by both CD133⁺ and CD133⁻ cells [53]. Similar results were obtained using NCAM⁺ and NCAM⁻ cells, raising questions about whether RP cells, regardless of specific identity, promote tissue repair similarly to differentiated cells [15]. Another interpretation of these findings is that more detailed identity markers are required, to have identity and efficacy correlate. However, a deeper look into the Methods section suggests that more than identity markers are different: cell source, selection, and culture are not consistent across studies. In our opinion, it is extremely important to account for these methodological differences, because they may lead to functional differences in ability to rescue diseased kidneys. Specifically, it is evident that the CD133⁺ cells used by Santeramo are

different than those used by Bussolati or by Romagnani [54, 55]: First, cell sources are different (infant kidneys vs. the tubular component of adult kidney vs. nephrectomy tissue vs. urine). In addition, the CD133⁺ cells are either sorted out from cell culture or expanded as an unselected cell population, with cell–cell signaling during expansion certainly impacted by these methodological differences. Many different cell types can express CD133, meaning that again a single marker is probably insufficient to identify a particular stem cell type. Second, CD133⁺ cells are also usually positive for the renal-specific marker, CD24. (Santeramo and Bussolati, unlike Romagnani, do not select specifically for CD24.) Together these reports suggest that CD133⁺/CD24⁻ cells are different from, or a specific subset of, cells identified by CD133⁺ alone. As in other aspects of preclinical stem cell studies, it is extremely important to consider the relationship of passage number to the phenotype of cells.

The same issues are important in comparing stem cells derived from amniotic fluid (AFSCs). In this case, the gestational age is an important variable. We derive AFSCs from discarded amniocentesis samples during the first trimester; others collect cells at delivery, obtaining fresh fluid samples but late in gestation [37]. We also believe that clonal isolation of the AFSCs is important for limiting heterogeneity of the cell product. We used a clonal population of AFSCs after positive selection for c-kit. Other groups use AFSCs derived from colony-forming units or with no selection for any markers [37]. These methodologies for source and derivation of AFSCs could account for the resultant differences in the final product population and functional differences in repair capacity.

The derivation and isolation of AdMSCs are complicated by disparate sources of adipose tissue. For example, AdMSCs derived subcutaneously express higher CD10 levels, whereas cells from visceral adipose express higher CD200 levels [56]. AdMSCs are extracted from processed lipoaspirates, usually involving collagenase digestion, and then sorted from a SVF. AdMSCs also may not emerge until after extensive culture of the SVF. In one study, the unsorted SVF had a similar therapeutic effect to AdMSCs in a renal I/R injury model [30], but further comparisons of all these cell types are warranted.

Recognizing that MSCs were heterogeneous populations and differed between labs, the International Society for Cellular Therapy (ISCT) recommended basic standards for phenotypic and functional characterization of these cells [57]. By ISCT definition, MSCs are plastic-adherent; identified by CD73⁺, CD90⁺, CD105⁺, CD11b⁻/CD14⁻, CD19⁻/CD79a⁻, CD34⁻, CD45⁻, and HLA-DR⁻; and can differentiate into osteoblasts, adipocytes, and chondrocytes. Although this helps to delineate an identity of MSCs, cells with this identity are still enormously heterogeneous within and between populations and may contain pro-inflammatory and expected anti-inflammatory phenotypes [58]. Recognition of the problem of ongoing heterogeneity led to development of immune functional assays for MSC as potency release criteria by ISCT [59].

MSC populations used in the clinic to date are not clonally derived. For autologous therapies, identity is often not defined. For nonclonal allogeneic, MSC identity and quality of these cells must be evaluated for every derivation to ensure safety and for correlation of identity and behavior [60]. Human ESC-derived MSC may be useful to generate a better characterized MSC population as well as for extending their

proliferative potential during manufacture and have been used successfully in preclinical lupus nephritis [61]. MSC exosomes also have therapeutic potential in the treatment of renal disease [62]. Because of their wide study, differences between MSC derived from different organs have been reported, but not yet compared with the much less studied, nestin-positive MSC isolated from kidney [19].

Translation of autologous therapies is inherently difficult because of the enormous heterogeneity of (outbred) humans, such that autologous MSC products are by nature different from person-to-person, contributing to the variability in therapeutic effects. Furthermore, in many instances, human RP cells are obtained from nephrectomized kidneys, [54] which can be expected to be disease with a pathological microenvironment (itself highly variable from patient to patient). Therefore, source (including age and sex of the donor and disease state), method of isolation and method of cell culture are all details that need harmonization to promote stem cells as therapeutic tools. Importantly, a better understanding of preclinical models of (acute) kidney disease is also dependent on developing a better understanding of human AKI [63], pointing to the importance of continuous interaction between preclinical and clinical researchers

Preclinical Animal Models of Disease

Another important aspect that can account for differences in results and their interpretation is the animal model. A variety of different methods are available to model clinical AKI, including some with direct clinical homologues including I/R injury, and renal toxic drugs (gentamycin, cisplatin, amphotericin). Glycerol is used to generate a rhabdomyolysis AKI model. Although all of these methods generate a form of AKI, the amount of damage is different between models, and each activates distinct molecular and cellular injury and regenerative pathways. Importantly, not all animal strains of a species respond to renal toxic interventions in the same way. For example, the 129 mouse strain is highly resistant to AKI (induced by I/R) versus C57BL mice [64].

Various preclinical models of CKD have been used to mimic the myriad human CKDs. Although reproducing common hallmarks of CKD—loss of renal function and the disruption of the glomerular filtration barrier (with loss of podocytes, the key cell for renal filtration)—the pathophysiology of diabetic nephropathy compared with, for example, focal segmental glomerulosclerosis is quite different. Inherited CKDs, including Alport Syndrome [65] or Pierson Syndrome [66], have yet other patterns of glomerular injury, interstitial fibrosis, and chronic inflammation.

The comparison of different stem cell types in the same experimental model is important to advance the field, so that the optimal, most potent cell type can be chosen for a particular renal pathology. In a cisplatin model of AKI, comparison of MSCs from allogeneic rat bone marrow, human adipose tissue, and human amniotic fluid showed differences in potency and also differences in the kinetics of the therapeutic effect [67].

Identifying an animal model that fully recapitulates human kidney disease and reflects the human response to stem cell therapy is a problem across all of translational medicine and not likely to be solved soon, pointing to the need to examine engineered organ models [68] in parallel with clinical studies, as well as mathematical modeling of disease [69, 70] and regeneration.

Route of Administration, Organ Localization, Survival and Toxicity

Several receptor–ligand systems are involved in the homing of stem cells to diseased organs. Expression of stromal cell-derived factor (SDF) and hyaluronic acid by damaged tissue are involved in stem cell recruitment; stem cells expressing chemokine receptors follow a SDF gradient to diseased tissue [70, 71]. Increased vascular permeability that follows inflammation may also contribute to tissue localization of administered cells. In addition to pathology-associated homing patterns, migration and localization of stem cells are dependent on the route of administration. Stem cells can be injected by many different routes (Table 1) and depending on route of delivery, may localize or become trapped in non-target organs, such as lungs or liver. Obviously, localization following tail vein injection will be different than after injection into the renal artery. In the first case, stem cells circulate systemically before reaching the kidney. Therefore, stem cell biodistribution is important to study when evaluating the potency and efficacy of a stem cell therapy: The numbers and precise localization of cells in the target organ are fundamental factors in renoprotective effects.

Stem cells have different capacities for integrating into target organs, as we showed with AFSC [37]. We found almost no integration in a CKD model (after 5 days) and very low integration in ATN models despite a positive therapeutic signal. Various approaches to enhancing integration of stem cells may lead to more potent therapies, depending on stem cell type. Feng et al. showed that modification of AFSCs with SIRT3 increased engraftment [72]. Others [73] suggest that AFSCs transfected with GDNF promotes differentiation into endothelial cell lineages.

Survival after injection is a critical factor in stem cell therapies; survival is often challenging in a toxic disease microenvironment. Data from a multitude of animal and clinical models show a wide variety of survival kinetics, complicated by the lack of easy-to-use, non-toxic, and inexpensive methodologies to track cells after injection. Some studies report a short-term integration, others reported a longer residence and these results are certainly dependent on stem cell type, on route of delivery, and details of the target pathology. Another generality important issue across cell types is that cells delivered in suspension are subject to anoikis if they do not quickly anchor, hence the development of 3D or patch grafts for stem cell therapeutics. It is difficult to follow cell survival in humans of course, because repeat biopsies are not possible. For MSC, across models and routes of delivery, the cells are usually cleared rapidly from the body, although therapeutic effect can outlast residence time, which has implications for understanding their mechanism of action. At this point, there are not much data to determine whether a prolonged time of survival time enhances renal regenerative response to a particular cell therapy. Nonetheless, embedding cells in a matrix [74] can enhance their survival (preventing anoikis), residence time, and so such engineered adjuncts of therapy are under active investigation (but beyond the scope of this review). Many other approaches have been proposed for *in vitro* manipulations to prepare cells before delivery to increase survival including statins [75] and hypoxic preconditioning [76]. These methods also require standardization so that results can be compared across studies.

Persistence of stem cell activity may require repetitive dosing, especially likely for CKDs. Very few papers describe the effects of multiple injections *in vivo*, and initial clinical trials are usually designed around safety of a single dose. It is also important to document the kinetics of kidney dysfunction and repair after each injection, which is likely to be different from injection to injection. Furthermore, although not widely recognized the immune response may be primed after a first MSC injection, resulting in alloimmunization [77] with potential loss of therapeutic efficacy.

Safety and toxicity of cell therapies is difficult to translate from animal to human models because of the model constraints. Often when human cells are studied, the animal models are immunodeficient, limiting insights into pathological inflammatory responses to the cells. Although MSCs are transplanted across allogeneic or even xenogeneic barriers, some animal studies have clearly shown that cellular and humoral responses against xenogeneic MSCs can develop in immunocompetent recipients [78]. Mobilization, reprogramming, or other manipulations of stem cells can trigger unintended events that carry risk for recipients. Cell culture conditions can contribute to toxicity. For example, a senescent population of cells can emerge in an over-passaged line, resulting in cells that behave differently, even antithetically, to the original population [60]. Expansion of pluripotent stem cells is associated with acquisition of mutations, including those in the gene encoding the tumor suppressor P53, leading to a recommendation that genetic characterization of these lines and their differentiated cell products be carried out before clinical application [79]. Adult (nonpluripotent) stem cells can also acquire chromosomal abnormalities with expansion [80] and so must be monitored for these changes during cell manufacturing processes used to generate working and master cell banks.

The risk of tumorigenesis must be considered in any application of stem cell therapy. Given the extensive experience with MSCs, tumorigenesis is not considered a risk of these therapies. Some stem cells express markers also found in cancer stem cells or upregulated in tumors. One example is the expression of CD133 in clear cell renal carcinoma [81], but the application of CD133⁺ renal stem cells has not been associated with development of this cancer.

The tumorigenic effect of naïve, undifferentiated iPSCs or RPs derived from them are not really clear, as this is a relatively new area of study, but iPSCs are defined by the ability to form teratomas. Human iPSCs directed toward a nephrogenic intermediate mesoderm were effective in ameliorating murine AKI (administered via tail vein) without apparent tumorigenesis [40]. Osafune used iPSC-derived renal tubulogenic progenitors to reduce tubular necrosis and dilatation, and interstitial fibrosis in a mouse I/R AKI model [41] without tumorigenesis. This same group recently reported a selectable marker method (CD9⁻CD140a⁺CD140b⁺CD271⁺) to enrich human iPSCs for RPs [82]. A recent report of a protective effect of undifferentiated naïve iPSCs administered into renal parenchyma in a rat 5/6 Nx model confirmed that undifferentiated iPSC have potential to generate tumors; treated animals developed tumors similar to Wilms' tumor [83]. Concentrated iPSC-conditioned medium, however, was shown effective in protecting from ischemic damage, and this acellular product was not tumorigenic [84].

The impact of aging and of the underlying chronic disease itself on donor stem cell function is an obvious constraint of

autologous therapies but deserves consideration for allogeneic therapies [78]. In the lab, donor cells are usually derived from healthy, young donors. Autologous therapy using old or diseased stem cells may have more limited therapeutic benefit for many reasons including reduced numbers, proliferation, differentiation capacity, and changed secretome profiles. Chronic inflammation, diabetes and uremic toxins also affect the host microenvironment [32] in kidney disease. The use of markers of senescence, such as telomerase, may be helpful in phenotyping therapeutic cells. Along the same lines, diseases that primarily affect older humans are usually studied in young animal models, which may overestimate the potency of the therapeutic effect.

These considerations are highlighted by a large multicenter international trial of MSC targeting AKI after cardiac surgery. This well-designed study of 156 subjects showed no benefit of MSC in shortening time to renal recovery, need for dialysis, or mortality. In fact, although not statistically significant, subjects treated with MSC had worse outcomes [85]. The authors cited many reasons why MSCs failed to show benefit including timing of the MSC treatment (late rather than preventive), the multifactorial underpinnings of AKI after cardiac surgery, longer bypass times in the MSC-treatment group, and pre-existing renal dysfunction in the study population. They did not note the important fact that cardiac surgery patients are usually old, often with hypertension and/or diabetes, and this phenotype is virtually never reflected in the usual animal models of I/R injury using young animals without pre-existing chronic vascular risk factors that damage kidneys over time.

Reagents Used to Study Stem Cell Biology: Interpretation of the Data

The FDA requires that reagents used in isolation, expansion, and manipulation of cells for therapeutic use are strictly defined to assure consistent quality and safety of the final cell product. We believe that the choice of reagents in analysis of cells during preclinical development is also extremely important and often under-emphasized. For example, interpretation of the results (specifically related to stem cell integration) can be difficult if cell surface and membrane dyes are used. These dyes are diluted over time by proliferation of the labeled population or if they fuse to host cells, limiting interpretation of biodistribution studies.

Antibodies for western blots are also a huge problem throughout biology. In our hands, for example, evaluating *de novo* deposition of collagen IV α 3, α 4, α 5 within the glomerular basement membrane by injected stem cells was challenging, as we found only one antibody that distinguishes this collagen trimer. Protocol adjustments and accurate interpretation of the results require knowing if the antibody is directed to a surface versus an intracellular epitope. Secondary antibody fluorescent labeling and its detection by flow cytometry can layer additional complexity onto data interpretation. The fluorescent signal on a flow cytometer must be gated and defined by thresholds that can be very nuanced, especially when the population of interest is rare. Variation between machines, the quality of the fluorochrome (between manufacturers and lots), and the binding affinity of the secondary antibody to the primary antibody will alter detection and analysis by flow cytometry.

In summary, we believe that cell source including quality of the sample, age, sex and disease status (phenotype) of the

donor, and so forth, methods of cell isolation and culture, pre-clinical models, route of administration, biodistribution, survival, toxicity, and reagents used during pre-clinical analysis all require detailed consideration, documentation, and ideally harmonization, to advance stem cell therapies. The quality measures required for clinical application should be adapted as much as possible during preclinical development of stem cell products.

STEM CELLS AND CLINICAL TRIALS FOR RENAL DISEASE

Stem cells (from any source) are an incredible research tool for understanding kidney regeneration. Once injected *in vivo*, they can serve as a cellular “indicator” of markers of disease progression and regression, by comparing molecular and histological changes before and after their delivery. On the other hand, when stem cells are being developed for therapies, there is less emphasis on the mechanisms of action and more on quantifying clinical endpoints of therapy.

Advancing Clinical Trials of Stem Cells for Renal Disease

Most stem cell clinical trials for renal disease use MSCs, which are not truly regenerative therapies. As noted earlier, MSCs differ qualitatively from person-to-person and between labs and manufacturers, in addition to the heterogeneity inherent in different tissue sources (marrow, adipose, etc), and heterogeneous kidney diseases are studied. It is therefore not possible to do a rigorous meta-analysis of MSC trials for renal disease [60], but some of the details of these studies hold important lessons.

The first trials using bone-marrow derived MSCs suggested that these cells could be given safely. In cardiac surgery patients, at high risk of post-operative AKI, MSC administration was safe and well-tolerated [86]. Autologous AdMSCs given via the renal artery to patients with renovascular disease were safe and improved renal blood flow and oxygenation 3 months after treatment ($n = 14$) compared with medical therapy alone ($n = 14$) [87]. In the first placebo controlled, two-dose trial of allogeneic BM-derived mesenchymal precursor cells for patients with diabetic nephropathy (still with only $n = 10$ /group), cells were again safe and did not elicit an immune response; this underpowered study suggested a trend toward a therapeutic effect at 12 weeks [88]. A study of six autosomal dominant polycystic kidney disease patients given autologous BM-MSCs intravenously again confirmed safety of these cells, but renal function was not improved 1 year after therapy [89]. A study of 30 patients with heterogeneous CKDs including 10 renal transplant patients suggested renal function improvement at 6 months after autologous BM-MSC [90].

Complications have occurred with these “benign” cells. Administration of umbilical cord-derived MSCs to two renal transplant patients was complicated by thrombosis of the peripheral vein injection site [91], likely a function of inadequate prevention of cell clumping. Another case report suggested that AdMSCs worsened renal function in a patient, whose CKD had been stable. In this case, renal biopsy suggested a massive inflammatory response including cells expressing surface markers of the presumed stem cell product [92]. As noted earlier, a uniquely large and well-designed international study of MSC for AKI in the context of cardiac surgery was halted when the treated group appeared to fare worse than untreated controls [85].

In summary, MSC clinical trials target a wide variety (and stage) of kidney diseases; trials are generally small, so that the clinical benefit of MSC therapy for AKI or CKD has not yet been demonstrated. Only a few studies have long follow-up: A study of autologous BM-MSCs in 30 CKD patients showed benefit to renal function [93] out to 18 months. Together a review of these studies confirms our bias that it will be necessary to reduce the confounding variables (in cells, preclinical models, and human disease phenotyping) that contribute to difficulty interpreting and comparing clinical trial results. Often, for proprietary reasons, details needed to compare clinical studies rigorously, are simply not available to researchers.

Standardized Stem Cell Protocols: Available Repositories of Different Cell Sources

Standardized cell lines (and derivation and characterization protocols) could be useful for data pooling by the research community, perhaps under the auspices of a national funding agency. Although challenging to execute, standardized lines and protocols would ultimately benefit the research community and patients, although may be opposed by biotechnology companies competing in this space with proprietary lines. For now, peer-reviewed journals should provide adequate space to present key biological authentication statements (as requested in NIH applications). Room for detailed supplemental protocols with specific focus on reagent details used for cell isolation and culture, in addition to a very detailed description (especially for human cells) of donor sex/age and exclusion/inclusion criteria will be helpful for interpreting results between labs. Information about population doubling (and time) at the time of preclinical application should be provided, along with cell density at passage, clonal versus nonclonal generation, and culture media protocols. These very tedious details are often missing in the literature. Nevertheless, this information is extremely critical to facilitate a smooth transition from academic labs to commercial manufacturing.

Recently, the need for a standard MSC ruler whose purpose would be to “serve as a common calibration tool and provide a central data point” was raised [94]. This interesting approach requires the definition of reference cell material needed to generate baseline characterization data (phenotype; karyotype; gene expression profile; cytokine secretion profile; and functional read-outs) to be set as general common reference. Different sources will likely contribute to determine the standard MSC cell ruler, including data collection from large sets of cell materials, single-donor cell lines from cell banks, immortalized cell lines, and iPSC-derived self-renewing lines [95]. Once the standards are defined, ongoing management of a repository will require further funding and oversight by a qualified agency, but it is important for moving the field forward. A central cell bank available to all researchers would also help to make the results from different labs comparable, and so synergize research efforts. To this end, the NIH established a bank of clinical grade marrow-derived stromal cell products in 2012 [96]. The article describing the bank has only been cited 18 times, suggesting that the bank has not been used widely as a resource, despite its availability. Another suggestion is to identify optimal human pluripotent cell lines for use in preclinical kidney disease studies. Individual embryonic stem cell lines have marked differences in propensity to differentiate down a specific lineage [97] as do iPSC lines. Given the

numbers of iPSC banks, it may be possible to identify a line with high propensity to generate RPs for study in a number of kidney disease models across different labs.

Stem cell populations (like MSCs) behave differently in different disease contexts, so that efficacy and potency testing must be application specific. Molecular characterization of stem cells and their preclinical efficacy do not predictably translate into clinical benefit. Co-culture of the therapeutic stem cell product with specific renal cell types, and in mixed lymphocyte reactions, is useful for further characterizing the stem cells beyond expression analysis. Standard culture tests that may also inform mechanism of action include the induction of tubular epithelial cell proliferation, survival in co-culture, or reduction of lymphocyte proliferation. For example, an in vitro model of IL-10 release from blood cells has been suggested as a potency assay for MSC immunomodulation [98]. For some applications, the immunomodulatory function of MSCs can be assayed using their modulation of a cytokine signature in blood mononuclear cells [99, 100]. Co-cultures can be designed based on anticipated mechanisms of action, such as tube formation assays if vasculogenesis is important in the therapeutic response.

Standard Preclinical Small and Large Animal Models

No animal model completely recapitulates human disease. Furthermore, the precise pathophysiological features of various animal models of kidney disease require different endpoints for analysis, and the animal model often constrains the numbers of endpoints that can be examined, especially for histopathological endpoints requiring biopsy or necropsy. For mouse models, frequent blood sampling is difficult; surgical manipulation, considering size alone, is also technically challenging; and miniaturized continuous physiological monitoring is expensive and not readily available. For these reasons and because murine disease often does not capture the heterogeneity of human disease, mouse models are usually insufficient alone for justifying advancement to clinical application, especially of a novel therapy. In addition, renal structure and function (physiology) differ among species and strains, and caution is warranted when extrapolating experimental data from one animal model to another (and to humans). For these reasons, large animal models remain important for translation and should be characterized as deeply as possible by experts in renal physiology and veterinary disease. Large animal models, until more sophisticated virtual models are available, are necessary to aid in establishing dose, scalability, safety and toxicity, and route of delivery. We realize that using larger animal models is complicated and expensive, but strong collaborations between

different groups of experts (researchers and veterinarians) can improve the cost:benefit ratio of large animal studies.

The best animal models for acute and chronic kidney diseases need to be further characterized for the research community. For example, researchers using a strain of mice in a particular disease must understand the kinetics of disease including blood markers, proteinuria, progression of histological damage in the tubules versus glomerulus, and so forth, in the untreated disease in that particular strain. Ideally comparative detailed studies of AKI and CKD models between strains could move the field forward, but funding for these kinds of studies is difficult to obtain. A select committee of experts (researchers, clinicians, statisticians, etc.) to develop best practices for preclinical study design, including stem cell source and disease model, and set standards for methods of isolation, culture, administration, analysis, could be better fostered by professional societies and/or funding agencies.

CONCLUSION

An enormous body of preclinical literature supports the application of stem cells to treat renal disease, but no safe, scalable and effective therapies have yet emerged from this research effort. To get through the current research bottleneck, we believe that organized collaboration of the research community—with help from professional, standards and funding agencies—is necessary, with the goal of developing harmonized protocols for isolation, characterization, and culture of stem cells, as well as full characterization of preclinical models of kidney disease. Collaboration and harmonization are necessary changes to the research culture, which should synergize experimental efforts of individual groups by making data comparable from lab to lab, enhancing statistical power, to help speed translation of stem cell therapies for the major unmet need in kidney diseases.

AUTHOR CONTRIBUTIONS

J.M.: data analysis and interpretation, manuscript writing; B.B.: data analysis and interpretation, manuscript writing; M.C.: conception and design, manuscript writing; L.P.: conception and design, manuscript writing, and final approval.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declared no conflict of interest.

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