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# The Promise of Mesenchymal Stem Cell Therapy for Diabetic Kidney Disease

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**Abstract** Diabetes mellitus (DM) commonly leads to progressive chronic kidney disease despite current best medical practice. The pathogenesis of diabetic kidney disease (DKD) involves a complex network of primary and secondary mechanisms with both intra-renal and systemic components. Apart from inhibition of the renin angiotensin aldosterone system, targeting individual pathogenic mediators with drug therapy has not, thus far, been proven to have high clinical value. Stem or progenitor cell therapies offer an alternative strategy for modulating complex disease processes through suppressing multiple pathogenic pathways and promoting pro-regenerative mechanisms. Mesenchymal stem cells (MSCs) have shown particular promise based on their accessibility from adult tissues and their diverse mechanisms of action including secretion of paracrine anti-inflammatory and cyto-protective factors. In this review, the progress toward clinical translation of MSC therapy for DKD is critically evaluated. Results from animal models suggest distinct potential for systemic MSC infusion to favourably modulate DKD progression. However, only a few early phase clinical trials have been initiated and efficacy in humans remains to be proven. Key knowledge gaps and research opportunities exist in this field. These include the need to gain greater understanding of in vivo mechanism of action, to identify quantifiable biomarkers of response to therapy and to define the optimal source, dose and timing of MSC administration. Given the rising prevalence of DM and DKD worldwide, continued progress toward harnessing the inherent regenerative functions of MSCs and other progenitor cells for even a subset of those affected has potential for profound societal benefits.

**Keywords** Diabetes mellitus · Diabetic nephropathy · Stem cells · Mesenchymal stem cells · Inflammation

## Introduction: Diabetic Kidney Disease and Its Pathogenesis

Diabetes mellitus (DM) is a global pandemic [1]. An estimated 171 million people worldwide had a confirmed diagnosis of DM in 2000 [2], doubling to 346 million in 2012—the majority having type 2 DM [3]. By 2030, this number is expected to increase to over 430 million people [4]. Key contributory factors include population ageing, increasing levels of obesity and declining physical activity coupled with improved life expectancy due to advances in medical care [1].

Consequent to this global pandemic, there has been an increase in the macro- and micro-vascular complications associated with DM, particularly diabetic nephropathy and other forms of chronic kidney disease (CKD), referred to in this review under the umbrella term of diabetic kidney disease (DKD) [5, 6]. Currently, DM is the commonest cause of

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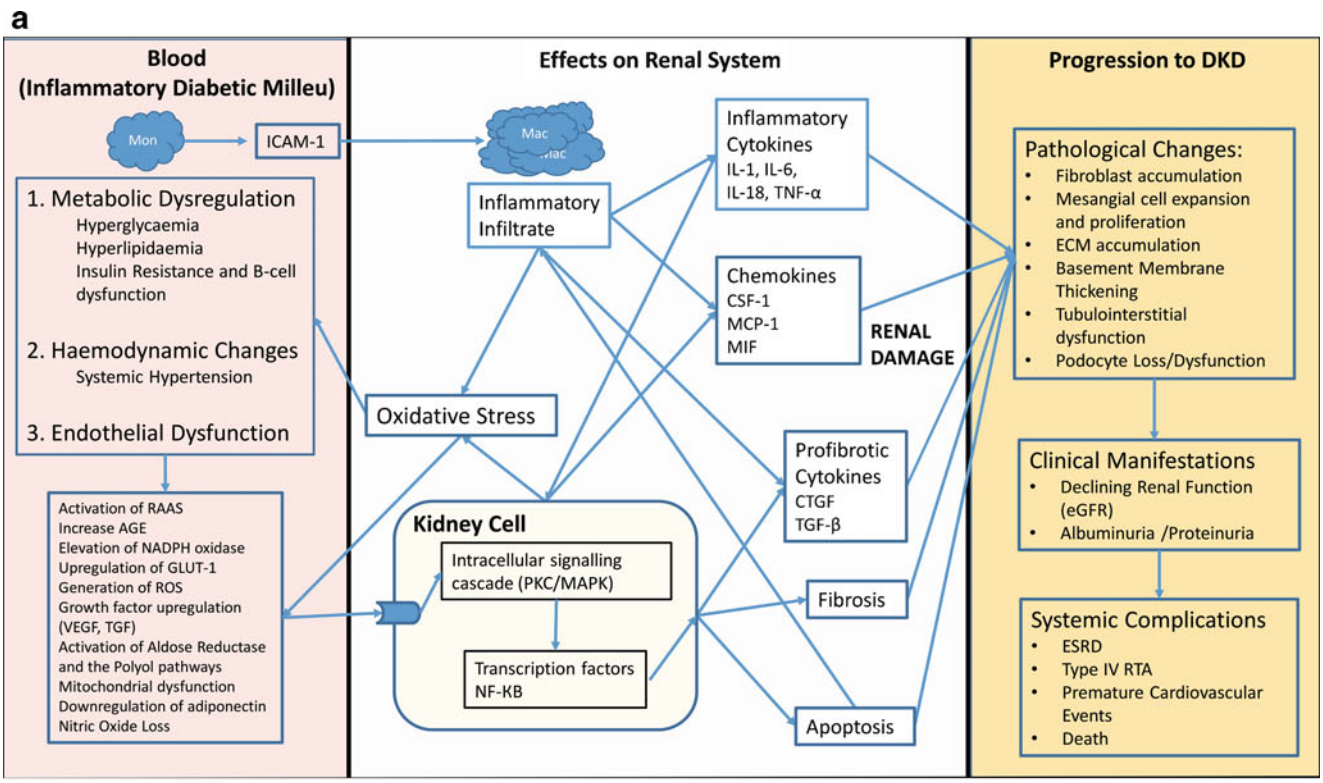
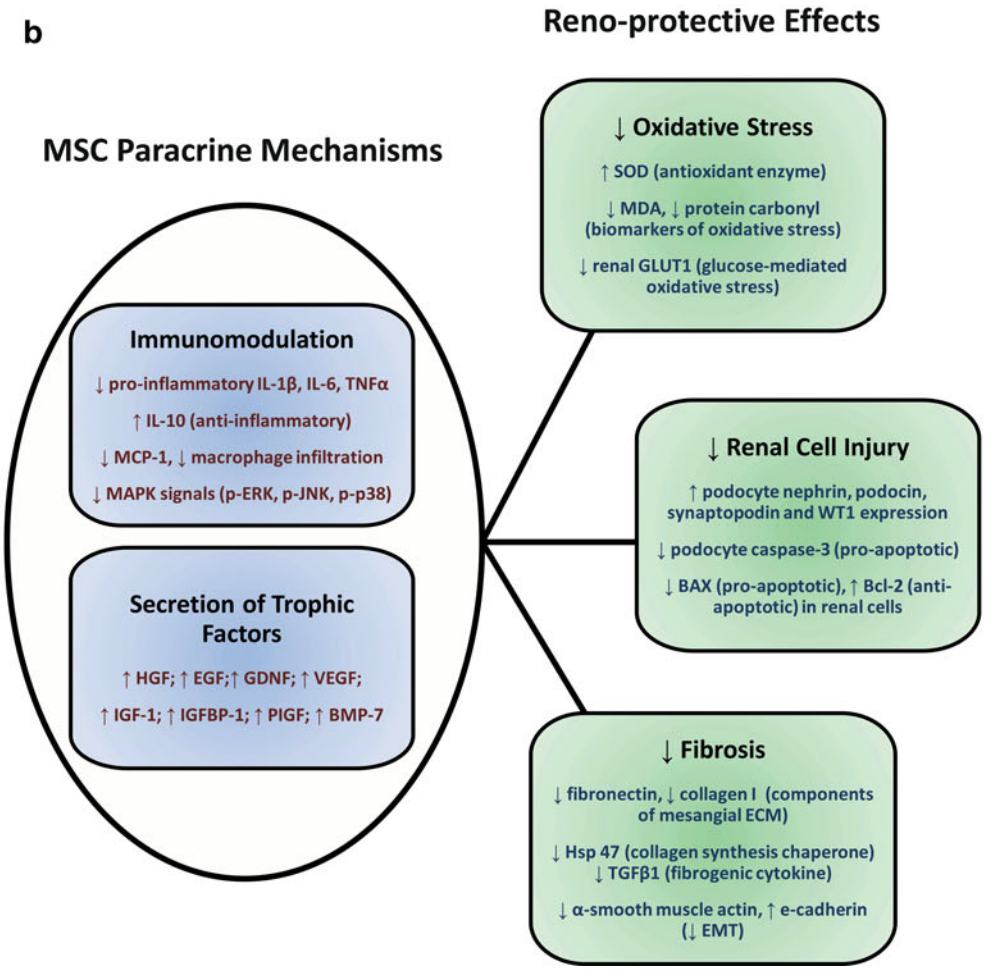

a
Blood
(Inflammatory Diabetic Milleu)
Mon
ICAM-1
1. Metabolic Dysregulation
Hyperglycaemia
Hyperlipidaemia
Insulin Resistance and B-cell dysfunction
2. Haemodynamic Changes
Systemic Hypertension
3. Endothelial Dysfunction
Activation of RAAS
Increase AGE
Elevation of NADPH oxidase
Upregulation of GLUT-1
Generation of ROS
Growth factor upregulation (VEGF, TGF)
Activation of Aldose Reductase and the Polyol pathways
Mitochondrial dysfunction
Downregulation of adiponectin
Nitric Oxide Loss
Effects on Renal System
Mac
Inflammatory Infiltrate
Oxidative Stress
Kidney Cell
Intracellular signalling cascade (PKC/MAPK)
Transcription factors NF-KB
Inflammatory Cytokines
IL-1, IL-6,
IL-18, TNF-α
Chemokines
CSF-1
MCP-1
MIF
RENAL DAMAGE
Profibrotic Cytokines
CTGF
TGF-β
Fibrosis
Apoptosis
Progression to DKD
Pathological Changes:
Fibroblast accumulation
Mesangial cell expansion and proliferation
ECM accumulation
Basement Membrane Thickening
Tubulointerstitial dysfunction
Podocyte Loss/Dysfunction
Clinical Manifestations
Declining Renal Function (eGFR)
Albuminuria /Proteinuria
Systemic Complications
ESRD
Type IV RTA
Premature Cardiovascular Events
Death


b
Reno-protective Effects
MSC Paracrine Mechanisms
Immunomodulation
↓ pro-inflammatory IL-1β, IL-6, TNFα
↑ IL-10 (anti-inflammatory)
↓ MCP-1, ↓ macrophage infiltration
↓ MAPK signals (p-ERK, p-JNK, p-p38)
Secretion of Trophic Factors
↑ HGF; ↑ EGF;↑ GDNF; ↑ VEGF;
↑ IGF-1; ↑ IGFBP-1; ↑ PIGF; ↑ BMP-7
↓ Oxidative Stress
↑ SOD (antioxidant enzyme)
↓ MDA, ↓ protein carbonyl (biomarkers of oxidative stress)
↓ renal GLUT1 (glucose-mediated oxidative stress)
↓ Renal Cell Injury
↑ podocyte nephrin, podocin, synaptopodin and WT1 expression
↓ podocyte caspase-3 (pro-apoptotic)
↓ BAX (pro-apoptotic), ↑ Bcl-2 (anti-apoptotic) in renal cells
↓ Fibrosis
↓ fibronectin, ↓ collagen I (components of mesangial ECM)
↓ Hsp 47 (collagen synthesis chaperone)
↓ TGFβ1 (fibrogenic cytokine)
↓ α-smooth muscle actin, ↑ e-cadherin (↓ EMT)

◀ **Fig. 1** **a** A schematic representation of known elements of the pathophysiological networks involved in the development and progression of diabetic kidney disease (DKD) is shown. Abbreviations for **a**: *Mon* = monocyte; *ICAM-1* = intracellular adhesion molecule 1; *Mac* = macrophages; *RAAS* = renin-angiotensin aldosterone system; *AGE* = advanced glycation end-products; *NADPH* = nicotinamide adenine dinucleotide phosphate; *GLUT-1* = glucose transporter 1; *ROS* = reactive oxygen species; *VEGF* = vascular endothelial growth factor; *TGF* = transforming growth factor; *IL* = interleukin; *TNF* = tumour necrosis factor; *PKC* = protein kinase C; *MAPK* = mitogen-activated protein kinases; *CSF-1* = colony-stimulating factor 1; *MCP-1* = monocyte chemotactic protein 1; *MIF* = macrophage migration inhibitory factor; *CTGF* = connective tissue growth factor; *ECM* = extracellular matrix; *eGFR* = estimated glomerular filtration rate; *ESRD* = end-stage renal disease; *RTA* = renal tubular acidosis. **b** A conceptual model is shown of the mechanisms whereby paracrine properties of systemically administered MSCs may exert reno-protective effects in the setting of DKD based on insights from published animal model studies. Abbreviations for **b**: *SOD* = superoxide dismutase; *MDA* = malondialdehyde; *GLUT 1* = glucose transporter 1; *ECM*, = extracellular matrix; *Hsp 47* = heat shock protein 47; *TGFβ1* = transforming growth factor β1; *EMT* = epithelial mesenchymal transition; *Th1* = T helper 1 lymphocytes; *IL-1β* = interleukin-1β; *IL-6* = interleukin-6; *TNFα* = tumour necrosis factor α; *IL-10* = interleukin-10; *MCP-1* = monocyte chemotactic protein 1; *MAPK* = mitogen-activated protein kinase; *p-ERK* = phosphorylated extracellular signal-regulated kinase; *p-JNK* = phosphorylated jun n-terminal kinase; *p38* = phosphorylated p38 kinase; *HGF* = hepatocyte growth factor; *EGF* = epidermal growth factor; *GDNF* = glial cell line-derived neurotrophic factor; *VEGF* = vascular endothelial growth factor; *IGF-1* = insulin-like growth factor 1; *IGFBP-1* = insulin-like growth factor-binding protein-1; *PlGF* = placental growth factor; *BMP-7* = bone morphogenetic protein; *WT1* = Wilms tumour 1 protein; *BAX* = Bcl-2-associated X protein; *Bcl-2* = B cell lymphoma-2 protein

CKD and end-stage renal disease (ESRD) worldwide [7]. The reported prevalence of CKD amongst those with DM varies from 8.6 to 17.7 % [5, 8, 9] with higher prevalence among people of African American, Asian and Native American ethnicity compared to Caucasians [7, 10]. The widespread adaptation of the Modification of Diet in Renal Disease (MDRD) equation for estimating glomerular filtration rate (eGFR) may also have contributed to the reporting of increased DKD prevalence [11]. Importantly, DKD is associated with greatly increased risk for cardiovascular [12–14] and all-cause mortality [15, 16, 17••, 18•]. Individuals with ESRD secondary to DKD have an annual mortality rate of approximately 20 % [19•]. In the USA, the cost of treating a person with DM and CKD has increased more than 11-fold in the last decade [20].

The most consistent pathological features of DKD are capillary basement membrane thickening and diffuse and nodular glomerulosclerosis. In the early stages of DKD, these glomerular lesions manifest as hyper-filtration and increased albumin excretion followed, as the disease advances, by progressively increasing proteinuria and declining eGFR. Importantly, DKD is also associated with primary and secondary pathological changes to the vascular and tubulo-interstitial compartments, the severity of which exert a strong influence on the rate of loss of renal function. Furthermore, in a proportion of individuals with clinical features of DKD, additional primary renal conditions (e.g. IgA nephropathy, renal arterial disease) may be superimposed upon DM-driven pathological changes.

The pathophysiology of DKD (Fig. 1a) is primarily driven by elevated blood glucose but extends to a broad network of local and systemic processes [21••]. These processes remain only partially understood but key details have been revealed through experimental studies in cell culture, animal models, tissue samples and human subjects. For example, hyperglycaemia induces abnormal activation of protein kinase C (PKC) in renal parenchymal cells which is associated with up-regulation of the pro-fibrotic cytokine transforming growth factor-β (TGF-β) along with the matrix proteins fibronectin and collagen type IV, nitric oxide dysregulation, endothelial dysfunction and activation of the mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signalling pathways [22, 23]. Hyperglycaemia is also associated with high levels of advanced glycation end-products (AGE) which further stimulate the production of TGF-β.

Activation of the renin-angiotensin aldosterone system (RAAS) has been linked to hyperglycaemia-associated increased formation of succinate [24, 25]. This causes elevated levels of angiotensin II leading to pro-inflammatory signals, hypertrophy of mesangial and tubular epithelial cells, increases in TGF-β [26, 27] and monocyte chemoattractant protein-1 (MCP-1) [28, 29], and the generation of reactive oxygen species (ROS) [30]. Induction of MCP-1 results in increased trafficking of monocytes into the kidney. Infiltrating monocytes are then converted to macrophages which release pro-inflammatory factors including interleukin-6 (IL-6), tumour necrosis factor α (TNF-α) and ROS [31•]. During this inflammatory process, additional angiotensin II is also generated at tissue level by macrophages and lymphocytes [32].

Growth factors also contribute to the development of glomerular structural alterations. For example, elevated glucose levels induce an early activation of platelet-derived growth factor-β (PDGF-β) which causes an increase in TGF-β1 expression [33]. Systemic arterial hypertension and localised haemodynamic dysfunction may further exacerbate intrarenal inflammation and the production of growth factors and extracellular matrix (ECM) proteins.

Based on these and other pathogenic mechanisms, DKD is now understood to occur in the setting of a pro-inflammatory milieu that is driven by metabolic dysregulation [19•] and mediated by humoral factors that cause pathogenic structural and functional alterations to the kidney [34]. In addition to the primary parenchymal cells of the kidney, specific immune cells also act as an important source of pathogenic mediators in DKD. For example, the severity of glomerulosclerosis is associated with the extent of macrophage accumulation in the kidney [35, 36]. While the role of lymphocytes has yet to be clearly defined [37], early DKD is associated with an increase in activated T cells [38]. A counter-balancing role for

regulatory T cells (T-reg) has also been demonstrated in animal models of DKD. It has been shown that the anti-inflammatory effects of T-reg ameliorate metabolic abnormalities and insulin resistance [39], while depletion of T-reg exacerbates albuminuria and hyperfiltration [40].

It is clear from this brief synopsis of DKD pathophysiology that the driving factors, primary cell responses and secondary, exacerbating factors represent a complex network of damaging mechanisms that are unlikely to be reversed by targeting a single mediator or intracellular pathway. Cellular therapies, particularly stem or progenitor cells, offer the potential for modifying multiple pathogenic mechanisms simultaneously and for actively promoting inherent capacity for tissue repair and regeneration [41•]. In this review, we summarise and critically evaluate the evidence for mesenchymal stem cells (MSCs)—a progenitor cell population that can be culture-expanded to large numbers from samples of bone marrow, fat, umbilical cord and other human tissues—as a cellular therapy for DKD.

## Animal Models Used to Evaluate MSCs in DKD

Rodents have served as the primary animal model of DKD due to their widespread availability, well defined genotypes, large repertoire of associated experimental reagents and amenability to genetic modification [42]. Almost all in vivo studies of MSCs in models of DKD have been carried out in mice or rats. Recently, however, Pan et al. reported results of a study evaluating the effects of MSC administration on DKD in tree shrews—a species having greater genome homology with primates which may also develop spontaneous dysglycaemia [43–47]

The characteristics of a given model of DKD reflect both the method(s) used to induce DM and the species/strain susceptibility to the development of DKD [48]. A range of methods has been developed for inducing type 1- or type 2-like DM in mice, including dietary, pharmacologic and genetic interventions [49]. Pharmacologic induction of DM with streptozotocin (STZ), with or without accelerating factors such as high-fat diet [43], uni-nephrectomy [50•] or use of the non-obese diabetic (NOD) strain [51], has been the predominant rodent DKD model used in the evaluation of MSCs. Experimental results in the db/db mouse model of obese, type 2 DM with uni-nephrectomy have also been reported in abstract form [52]. Some concerns related to the renal disease observed in STZ-induced type 1 DM are of relevance. Non-specific renal cytotoxicity of STZ itself can lead to acute tubular injury raising the concern that some of the observed beneficial effects of MSC administration may be due to their well-documented capacity to reduce the severity of acute kidney injury (AKI) [41•, 53–55]. While all but one reported study used a low-dose STZ regimen [56], a residual risk of STZ-induced AKI has been described even at low doses [57]. Furthermore, STZ-induced diabetes rarely progresses to histologically advanced DKD and renal failure [58]. The recent development of murine models which more readily recapitulate the more advanced features of DKD may be of value for testing the pre-clinical efficacy of MSCs across the spectrum of DKD severity [59–61].

## Efficacy and Mechanism of Action of MSCs in Pre-clinical Models of DKD

Over the past decade, several groups have reported results of experiments in which therapeutic benefits of MSCs were evaluated in the small animal models of DKD described above. These pre-clinical studies have involved administration of autologous/syngeneic [56, 62, 63], allogeneic [43, 64, 65, 66•, 67, 68] and xenogeneic (human) [50•, 51, 69•, 70, 71] MSCs. Most studies employed MSCs of bone marrow origin but umbilical cord- [70, 71] and adipose-derived MSCs [50•, 62, 69•] have also been used. Systemic administration of MSCs via the intravenous route has been utilised in the majority of studies. Two studies employed intra-cardiac administration via the left ventricle [51, 68] while another reported local delivery via the left renal artery [67].

These published studies have generally provided evidence that MSCs ameliorate clinically relevant indicators of DKD severity including albuminuria; serum creatinine/urea; glomerular hypertrophy, mesangial expansion and sclerosis; podoctye number and foot process effacement; and tubular injury and interstitial fibrosis. Taken together, the results to date indicate that systemic administration of one or more doses of MSCs exerts beneficial effects on proteinuric DKD in small animal models regardless of tissue source and genetic/species compatibility. In keeping with MSC anti-inflammatory properties, a consistent observation in rodent models of DKD has been reduction in the intra-renal expression of key inflammatory mediators such as TNFα, IL-6 and IL-1β and reduced infiltration by macrophages [51, 62, 64, 66•]. These studies also provide insights into other key issues related to the clinical translation of MSC therapy to human subjects with DKD including impact on glycaemic control, bio-distribution and persistence of administered cells and mechanism(s) of action.

The potential for MSCs to improve blood glucose control after systemic administration may delay the progression of DKD independently of direct reno-protective effects [72]. Of 14 studies assessing the in vivo effect of MSCs in animal models of DKD, 8 reported a concomitant significant reduction in blood glucose [43, 51, 62–65, 66•, 73]. One study reported a reduction in blood glucose only when allogeneic MSCs (allo-MSCs) were administered in combination with cyclosporine [68], suggesting that

immunologic rejection could hinder the propensity of allo-MSCs to lower blood glucose. Three of these nine studies reported concomitant islet cell regeneration despite low levels of MSC engraftment in the pancreas [51, 63, 73], suggesting that paracrine mechanisms underpin the anti-hyperglycaemic benefit of MSCs. This is in keeping with evidence from the wider literature regarding the beneficial effects of MSCs in models of DM [74–76]. For studies in which no significant reduction in blood glucose occurred [50•, 56, 67, 70, 71], various explanations have been proposed. Factors such as MSC tissue origin, administration route and dosage may influence their potency [72]. Additionally, variations in DKD model characteristics such as the mechanism and degree of pancreatic β cell injury at the time of administration may dictate whether rescue is possible [56]. Clarification of these factors will be paramount for maximising the efficacy of MSC therapy when applied to human DKD.

Regarding MSC bio-distribution in models of DKD, all studies have demonstrated tracking of MSCs to the kidneys after systemic administration, albeit of a degree and duration that is unlikely to fully explain their beneficial effects on renal function and structure [77]. Although Lee et al. reported direct differentiation of some human bone marrow-derived MSCs into intra-renal $CD31^+$ endothelial cells in diabetic NOD/scid mice [51], it is generally accepted that such trans-differentiation events are rare at best and insufficient to represent a major mechanism of tissue repair. However, Wang et al. also reported that 10 % of glomeruli contained adipocytes arising from administered MSCs in rats with type 1 DM [67], a finding which should be closely monitored in human studies.

It remains unclear whether trafficking of systemically administered MSCs to the kidney in DKD is essential for maximising the beneficial effects of their paracrine activities. Nonetheless, two recent studies have reported results for the application of ultrasound-targeted micro-bubble destruction (UTMD) to enhance MSC homing to diabetic kidneys. Zhang et al. demonstrated that UTMD increased MSC localisation to the kidneys by increasing interstitial capillary permeability and endothelial VCAM-1 expression. In this study, UTMD-enhanced renal trafficking was associated with reduced urinary albumin excretion without a concomitant reduction in blood glucose compared with MSC administration alone [73]. Subsequently, Wu et al. exploited the interaction between stromal cell-derived factor-1 (SDF-1) and the CXCR4 receptor, which is of central importance to MSC migration [78], by loading micro-bubbles with covalently attached SDF-1. The resultant increase in SDF-1 in the renal interstitium after application of ultrasound greatly enhanced MSC homing at 24 h compared to conventional UTMD [79•]. No studies have yet assessed the ability of UTMD to increase MSC homing to the pancreas [80].

A large body of literature has accumulated related to the mediators and pathways involved in MSC paracrine functions in diseases involving maladaptive inflammation and tissue degeneration [41•]. Figure 1b summarises the paracrine reno-protective effects of MSCs which have been elucidated in animal models of DKD to date. As shown, experimental evidence supports modulatory effects of MSC-derived factors on mechanisms of fibrosis, oxidative stress, immune/inflammatory activity, cellular de-differentiation pathways and growth factor responses. Rather than viewing them independently, it is important to recognise that most of these mechanisms interact at one or more levels within the complex molecular milieu of DKD. For example, Lv et al. demonstrated that hepatocyte growth factor (HGF) elaboration from MSCs significantly reduced hyperglycaemia-induced TGFβ1 expression in mesangial cells, which in turn was responsible for the reduced GLUT1 expression and consequent reduction in glucose-mediated intracellular oxidative stress observed with allo-MSC administration [65]. Despite this, the consequences of trophic factor secretion by MSCs in the setting of DKD remains under-evaluated, with the reno-protective effect of only three of these factors being rigorously examined using neutralising antibodies and/or small interfering RNA in vitro. Along with HGF, epidermal growth factor (EGF) has been shown to prevent podocyte apoptosis and hyperglycaemia-induced down-regulation of synaptopodin and nephrin expression [69•]. Glial cell line-derived neurotrophic factor (GDNF) prevents hyperglycaemia-induced down-regulation of podocyte synaptopodin and Wilms tumour 1 protein [50•]. Clearly, continued investigation of MSCs mechanism of action in animal models of DKD is needed to optimise clinical translation.

## Biomarkers of DKD Progression and Their Role in Clinical Translation of MSCs

A significant unmet need for successful translation of MSCs, and other cellular therapies for DM and its complications, in human subjects is the identification of measurable factors (“biomarkers”) that can serve as predictors or early indicators of favourable therapeutic response. This is particularly important for DKD as impact on the relevant “hard outcomes” such as rate of decline of GFR, development of ESRD, cardiovascular events or death may not be evident for years following intervention. Increased urine albumin excretion is an important marker of risk for development of progressive DKD [81–83] but, in recent years, it has been shown to fluctuate over time in many individuals with DM [83–86]. Thus, although albuminuria combined with

eGFR continues to be a guideline-endorsed biomarker of CKD [87], its value as a surrogate for future risk of progressive CKD/ESRD in the context of clinical trials in DKD is open to question. It is against this backdrop that the evaluation of alternative biomarkers or panels of biomarkers linked to a growing understanding of the pathophysiology of DKD has become an important research topic [88–90]. Although the majority of novel DKD biomarkers are currently in the early stages of validation, several have biological plausibility and have been the subject of promising studies in cohorts of subjects with diabetes. In some cases, such emerging biomarkers may be linked to the putative mechanisms of action of MSCs in animal models of DKD discussed above (see Table 1). Thus, the design of experimental systems and clinical trials of MSCs as a therapeutic intervention for DKD may be enhanced by careful consideration of the growing literature on measurable biomarkers in blood and urine. For the purpose of this review, we will focus on a selection of such novel biomarkers which we believe may have specific potential for predicting the effects of MSC therapy in DKD.

Pro-inflammatory cytokines may represent important indicators of DKD risk and severity. For example, serum and urine concentrations of TNF-α are elevated in people with DM compared to healthy controls [91–93]. Navarro et al. demonstrated that urinary TNF-α is raised in type 2 DM and independently correlates with albuminuria status and renal function [93]. In vivo models of the role of MSCs on renal function in rats with DKD have demonstrated a decrease in TNF-α following MSC therapy [64]. The receptors for TNF-α, TNF receptor-1 (TNFR1) or TNF receptor-2 (TNFR2), may also serve as indicators of DKD severity in their soluble forms (sTNFR1 and sTNFR2). Niewczas et al. in a cross-sectional study of type 1 diabetic subjects identified that serum TNF-α and the sTNFRs were associated with cystatin-C-based eGFR (cC-eGFR) in univariate analyses. However, on multivariate analysis, only the association with sTNFRs remained significant [94]. Further work by this group demonstrated that circulating TNFRs are predictive of stage 3 CKD in type 1 DM [95] and of ESRD in type 2 DM [96••]. Serum and/or urine concentrations of other pro-inflammatory mediators such as IL-6, IL-1β and MCP-1 have also been shown to be associated with DKD in patient cohorts. Although their value as clinical predictors of DKD progression and complications has not been robustly proven, they may also be considered as putative biomarkers of the anti-inflammatory effects of MSCs.

**Table 1** Potential biomarkers for monitoring MSC response in DKD

| Biomarker | Reference | Source | Key results |
|---|---|---|---|
| Individual biomarkers | | | |
| TNF-α | [91–94] | Serum + urine | Elevated in DM and DKD (macro- > micro- > normo-albuminuria) |
| TNFR1 + 2 | [94, 95, 96••, 130] | Serum + urine | Predictive of progression to CKD3 in type 1 DM and to ESRD in type 2 DM |
| Adiponectin | [101–113, 114•] | Serum + urine | Serum: predictive of progression in macro-albuminuria<br>Urine: predictive of progression to ESRD |
| NGAL | [117–121] | Serum + urine | Elevated in DM and DKD (increased with progressive albuminuria) |
| FGF-23 | [123, 130••] | Serum | Predictive of progression in macro-albuminuria |
| FGF-21 | [124, 130••] | Serum | Predictive of eGFR decline in normo-albuminuria and of progression of CKD3 |
| KIM-1 | [121, 125, 127–129, 130••] | Serum + urine | Serum: predictive of eGFR decline and ESRD.<br>Urine: predictive of eGFR decline. Lower levels associated with remission of micro-albuminuria. Reduced by ARB therapy |
| IL-6 | [150••] | Serum | Higher baseline level associated with stabilisation of eGFR in phase I/II trials of bone marrow MPCs |
| Biomarker panels | | | |
| Peptide and metabolite panels | [21••, 91, 144, 145, 146•, 147, 148] | Urine | Elevated in DKD. Predictive of progression to micro- or macro-albuminuria |
| Candidate biomarker panel | [130••] | Serum | Predictive of progression from CKD3 |

*TNF-α* tumour necrosis factor alpha, *DM* diabetes mellitus, *DKD* diabetic kidney disease, *TNFR1+2* TNF receptor 1 and TNF receptor 2, *CKD3* chronic kidney disease stage 3, *ESRD* end-stage renal disease, *NGAL* neutrophil gelatinase-associated lipocalin, *FGF-23* fibroblast growth factor 23, *FGF-21* fibroblast growth factor 21, *eGFR* estimated glomerular filtration rate, *KIM-1* kidney injury molecule 1, *IL-6* interleukin 6, *MPCs* mesenchymal precursor cells, *ARB* angiotensin receptor blocker

Type 2 DM is associated with down-regulation of the adipokine adiponectin in association with obesity and insulin resistance [97]. Adiponectin is reported to have a reno-protective effect in rodent experiments [98–100]. Consistent with this, serum adiponectin levels have been observed to be negatively correlated with urinary albumin excretion in subjects with DM, normo-albuminuria and preserved GFR. However, this correlation is less consistent in subjects with micro-albuminuria [98, 101–104]. Furthermore, in subjects with overt DKD, serum and urine adiponectin levels have been shown to have positive correlations with albuminuria and negative correlations with GFR [105–112]. Saraheimo et al., in a prospective study of subjects with type 1 DM, observed that increased serum adiponectin predicted progression from macro- but not micro- or normo-albuminuria to ESRD [113]. Similarly, Panduru et al. identified an association between urinary adiponectin and progression to ESRD in type 1 DM [114•]. The authors concluded that urinary adiponectin was a better predictor than albumin excretion rate. Thus, serum or urine adiponectin may be of specific value as a predictor of DKD progression in the setting of overt proteinuria and reduced eGFR—perhaps the most likely target group for MSC therapy.

Other novel biomarkers of interest include neutrophil gelatinase-associated lipocalin (NGAL), fibroblast growth factor 23 (FGF-23) and fibroblast growth factor 21 (FGF-21). NGAL is a small molecule belonging to the lipocalin superfamily which plays a role in apoptosis, immune regulation and transportation of small hydrophobic molecules [88, 115]. In a cohort of subjects with type 2 DM, Yang et al. demonstrated that urine NGAL correlated positively with cystatin C, urea nitrogen and serum creatinine and inversely with eGFR [116]. Nielsen et al. demonstrated that urine NGAL was elevated in type 1 DM with and without albuminuria suggesting a tubular source [117]. They showed that urine NGAL increases significantly with increasing albuminuria. In a study by Bolignano et al., urine and serum NGAL were higher in subjects with DM compared to controls and the rate of increase of NGAL was associated with increasing albuminuria [118]. However, controversy exists regarding the utility of this marker as other authors have suggested that there may not be an association following adjustment for clinical predictors [119–121]. FGF-23 is an osteocyte-produced hormone involved in the regulation of phosphate excretion and vitamin D activation [122]. Titan et al. demonstrated that serum FGF-23 concentration is an independent predictor of renal outcome in patients with type 2 DM and macro-albuminuria [123]. A related factor, FGF-21, is secreted by the liver and has been shown to regulate various metabolic conditions [124]. In a large cohort of subjects with type 2 DM, higher serum FGF-21 concentration at baseline was associated with eGFR decline during a median 4-year follow-up. In subjects with eGFR ≥60 mL/min/1.73m$^2$ and normo-albuminuria, serum FGF-21 was an independent predictor of eGFR decline. Other biomarkers such as kidney injury molecule-1 (KIM-1) [31•, 121, 125–129, 130••], vascular endothelial growth factor (VEGF) [131–135] and α-1 microglobulin [130••, 136–140] may also be relevant in trials of stem cell therapies.

As an alternative to measurement of individual biomarkers, assays involving quantification of biomarker panels may eventually allow for more precise prediction of adverse renal outcomes or responses to novel therapies. Recently, Looker et al. evaluated a large number of candidate biomarkers for their predictive value for rapid progression from CKD stage 3 in a longitudinally followed cohort of subjects with DKD [130••]. This study identified a minimal panel of 14 biomarkers which provided significant predictive value when added to clinical information. The panel included the above-mentioned KIM-1, FGF-21 and α-1 microglobulin along with other proteins and small molecules/metabolites. Building on rapid advances in mass spectrometry, urinary proteomics has been extensively applied as a technology for non-biased discovery of biomarkers panels for CKD/DKD progression [141–143]. Notably, Goode et al. identified a 273-peptide urine signature (CKD 273) with a sensitivity of 85.5 % and specificity of 100 % in classifying CKD among subjects from a multicentre prospective study [144]. Subsequently, the CKD 273 panel was shown to have predictive value for loss of renal function and death in a prospectively followed CKD cohort [145]. It was also validated as accurately identifying DKD [146•] and for predicting transition to micro- or macro-albuminuria in a cohort of subjects with type 2 DM [147]. In a longitudinal study of normo-albuminuric subjects with type 1 and type 2 DM, the CKD 273 panel significantly enhanced the predictive value of urinary albumin alone for development of macro-albuminuria up to 5 years later [148]. Sharma et al. quantified 94 urine metabolites using gas-chromatography-mass spectrometry and found that 13 metabolites were significantly reduced in subjects with DKD [21••]. The metabolic signature was specifically linked to mitochondrial metabolism and the authors concluded that global suppression of intra-renal mitochondrial function may be a measurable indicator of DKD severity. As MSCs and their products have been demonstrated to have direct cytoprotective effects on renal epithelial cells [149], it is interesting to speculate that changes in urine metabolites could serve as an early indicator of response to cellular therapy in DKD.

## Human Clinical Trials of MSCs in DKD

Clinical trial activity in the area of MSC therapy for DKD, or for other causes of CKD, has only recently been initiated and

remains at an early stage worldwide. A search of the major clinical trial registries [WHO International Clinical Trials Registry (www.who.int/ictrp/en/); EU Clinical Trials Register (www.clinicaltrialsregister.eu/) and the U.S. National Institutes of Health ClinicalTrials.gov (www.clinicaltrials.gov)] yielded only three clinical trials of MSC therapy in DKD as well as four additional trials in non-diabetic CKD. The details of these trials are summarised in Table 2. As is clear from the table, these clinical trials involve small numbers of subjects with DKD/CKD and represent either exploratory studies (Phase 0) or studies with safety as the primary end-point and potential signals of efficacy as secondary end-points (Phase I/II). Two clinical trials in DKD/CKD involve allo-MSC administration while the remainder focus on autologous MSCs from bone marrow or adipose tissue. With the exception of an ongoing trial of autologous MSCs delivered intra-arterially in subjects with reno-vascular disease, all trials involve intra-venous administration of various doses of MSCs.

To date, only one Phase I/II trial has been completed with results reported in abstract form at the 2015 American Diabetes Association national meeting [150••]. In this trial, two doses of a cell product manufactured by the Australian company Mesoblast Ltd. consisting of allogeneic bone marrow-derived Stro3+ mesenchymal precursor cells (MPCs) were compared with placebo infusion in a cohort of 30 subjects with type 2 DM and eGFR of 20–50 mL/min/1.73/$m^2$ on stable medical therapy. Preliminary results from 12 and 24 weeks follow-up indicated an acceptable safety profile for MPCs in the setting of relatively advanced DKD. In addition, trends for change in renal function provided an "efficacy signal" in that subjects receiving placebo had greater decline in eGFR during follow-up compared to those receiving cell infusion—particularly for those with higher baseline eGFR (>30 mL/min/1.73/$m^2$) and higher serum IL-6 concentration (>3.5 pg/dL). A Phase 0 trial of autologous bone marrow MSCs in 20 subjects with type 1 DM and nephropathy has completed enrolment in Iran. To our knowledge, results for this study have not yet been reported. Finally, the authors of this review, along with partner institutes from five other European countries (UK, Belgium, the Netherlands, Germany and Italy) have recently initiated a project (www.nephstrom.eu) which will conduct a multi-site, placebo-controlled, dose-escalating Phase I/II clinical trial of a prospectively isolated bone marrow-derived allo-MSC therapy in subjects with progressive, proteinuric DKD (eGFR 30–50 mL/min/1.73/$m^2$) despite optimal medical therapy (see Table 2). Thus, while it has been almost 10 years since the first promising animal model study [51], the clinical translation of MSC therapies for DKD is in its infancy and further development of the field may well be dependent on encouraging results from such early phase trials.

## Conclusion: What Are the Key Challenges and Unanswered Questions?

Of the many diseases for which MSCs are considered to be of potential benefit, progressive DKD represents one of the most significant worldwide health challenges. As described in this review, the therapeutic model which has been evaluated in pre-clinical models to date consists of a single, timed intervention by which multiple elements of disease pathogenesis are favourably modulated. The efficacy of this model rests predominantly upon MSC-associated paracrine mechanisms which result in alterations to the systemic and intra-renal milieu with consequent slowing or reversal of key pathogenic pathways including glomerular barrier dysfunction, pro-inflammatory cellular infiltration, tubular epithelial cell stress and progressive interstitial fibrosis. Thus, the clinical niche for MSC administration in DKD could be viewed as a broad re-programming of chronic nephrotoxic processes occurring in DM which may "reset the clock" of progression toward ESRD in responsive individuals. Of additional significance, MSC administration has also been demonstrated to improve glycaemic control as well as the advancement of other diabetic end-organ complications in some experimental settings. Furthermore, MSC therapy is conceptually compatible with established pharmacological and lifestyle-based treatments for DKD and with a model of intermittent administration that has proven to be highly effective for other "biological agents". However, as we make clear here, small animal models of DM and DKD provide, at best, only a partial reproduction of human disease pathogenesis and progression. Therefore, the design and outcome of early phase clinical trials in this area represent a critical juncture in the evolution of cellular therapies for diabetic complications. Confirmation of the safety of autologous and allogeneic progenitor cell therapy is paramount. However, the identification and interpretation of "efficacy signals" from these studies may well determine whether the necessary investment of funding, resources and expertise can be secured to proceed with trials of sufficient scope to robustly prove therapeutic value.

To conclude this review, we highlight the following areas for which ongoing and new research efforts will be needed to maximise the likelihood of widespread future clinical application of MSCs and other stem/progenitor cell therapies to DKD: (a) Further efficacy and mechanism of action studies in emerging animal models of DKD. (b) Development of in vitro systems and potency assays to optimise MSC production and patient selection for cell therapy intervention. (c) Identification of biomarkers linked to DKD progression and to in vivo MSC mechanism of action for application to Phase II/III clinical trials. (d) Economic analysis and modelling of the delivery, reimbursement and cost-effectiveness of MSC administration in DKD at varying stages of severity.

**Table 2** Summary of registered clinical trials of MSC therapies in DKD and non-diabetic CKD

| Trial number | Trial name | Condition | Registration | Cell therapy | Cell dose(s) | Route | Sponsor | Subject no. | Age range (years.) | Study type | Status | Trial Phase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NCT01843387 | Safety and efficacy of mesenchymal precursor cells in diabetic nephropathy | Diabetic nephropathy (type 2 DM) | ClinicalTrials.gov | Allogeneic bone marrow-derived MPCs | 150M or 300M | IV | Mesoblast Ltd, Australia | 30 | 50–85 | Placebo control: yes Randomization: yes Blinding: double-blind | Completed | 1/2A |
| IRCT2011112 91414N28 | Investigation on autologous mesenchymal stem cell transplantation in diabetic nephropathy (type one) | Diabetic nephropathy (type 1 DM) | WHO ICTRP | Autologous bone marrow-derived MSCs | 2M/kg | IV | Endocrinology and Metabolism Research Institute, Iran | 20 | 18–40 | Placebo control: no Randomization: no Blinding: no | Recruitment complete | 0 |
| NCT02585622 | Novel stromal cell therapy for diabetic kidney disease (NEPHSTROM) | Diabetic kidney disease (type 2 DM) | ClinicalTrials.gov | Allogeneic bone marrow-derived MSCs | 80M, 160M or 240 M | IV | Mario Negri Institute for Pharmacologic al Research, Italy | 48 | ≥18 | Placebo control: yes Randomization: yes Blinding: double-blind | Not yet recruiting | 1/2A |
| IRCT201204 248349N1 | Effects of mesenchymal stem cell therapy in patients with chronic kidney disease | CKD | WHO ICTRP | Autologous bone marrow-derived MSCs | 1M/kg | IV | Vice President for Science and Technology, Iran | 15 | 18–65 | Placebo control: no Randomization: no Blinding: no | Ongoing | N/A |
| NCT02166489 | Mesenchymal stem cells transplantation in patients with chronic renal failure due to polycystic kidney disease | CKD due to polycystic kidney disease | ClinicalTrials.gov | Autologous bone marrow-derived MSCs | 2M/kg | IV | Royan Institute, Iran | 6 | 18–60 | Placebo control: no Randomization: no Blinding: no | Ongoing | 1 |
| NCT02195323 | Autologous bone marrow derived mesenchymal stromal cells (BM-MSCs) in patients with chronic kidney disease | CKD | ClinicalTrials.gov | Autologous bone marrow-derived MSCs | 2M/kg | IV | Royan Institute, Iran | 10 | 40–60 | Placebo control: no Randomization: no Blinding: no | Ongoing | I |
| NCT02266394 | Hypoxia and inflammatory injury in human renovascular hypertension | CKD due to reno-vascular disease + ischemic nephropathy | ClinicalTrials.gov | Autologous adipose tissue-derived MSCs ± arterial stent | 0.2M/kg | IA | Mayo Clinic, USA | 42 | 40–80 | Placebo control: no Randomization: no Blinding: no | Ongoing | I |

*DM* diabetes mellitus, *MPCs* mesenchymal precursor cells, *M* million, *IV* intravenous, *WHO ICTRP* World Health Organisation International Clinical Trials Registry Platform, *MSCs* mesenchymal stem cells, *CKD* chronic kidney disease, *IA* intra-arterial

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**Compliance with Ethical Guidelines**

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- • Of importance
- •• Of major importance

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