Acta Diabetol (2014) 51:897–904
DOI 10.1007/s00592-014-0603-1

REVIEW ARTICLE

Harnessing the immunological properties of stem cells as a therapeutic option for diabetic nephropathy

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Received: 29 March 2014 / Accepted: 17 May 2014 / Published online: 4 June 2014

Abstract Diabetic nephropathy is the leading and possibly the most devastating complication of diabetes, with a prevalence ranging from 25 to 40 % in diabetic individuals, and as such represents an important challenge for public health worldwide. As a major cause of end-stage renal disease, diabetic nephropathy also accounts for a large proportion of deaths in diabetic individuals. To date, therapeutic options for overt diabetic nephropathy include medical interventions to reduce blood glucose levels and to control blood pressure and proteinuria. Recent evidence suggests a strong role for inflammation in the development and progression of diabetic nephropathy. Various immune cells, cytokines and chemokines have been implicated in the onset of diabetic nephropathy, while immune-related transcription factors and adhesion molecules have been correlated with the establishment of a renal proinflammatory microenvironment. Both inflammation and immune activation may promote severe distress in the kidney, with subsequent increased local fibrosis, ultimately leading to the development of end-stage renal disease. Stem cells are undifferentiated cells capable of regenerating virtually any organ or tissue and bearing important immunoregulatory and anti-inflammatory properties. Due to the aforementioned considerations, significant interest has been ignited with regard to the use of stem cells as novel therapeutics for diabetic nephropathy. Here, we will be examining in detail how anti-inflammatory properties of different populations of stem cells may offer novel therapy for the treatment of diabetic nephropathy.

Keywords Diabetic nephropathy · Stem cells · Type 2 diabetes · Type 1 diabetes

Managed by Massimo Porta.

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Introduction

Diabetic nephropathy (DN) is a complication of diabetes, characterized by a decline of glomerular filtration rate (GFR), with the possible increase in urinary albumin excretion (UAE) and the presence of tubular dysfunction in the absence of other kidney diseases [1, 2]. The appearance of macroalbuminuria (UAE $\geq$200 μg/min) has been implicated for years as a major determinant of DN; however, it is the reduction in GFR that leads individuals affected by type 1 (T1D) or type 2 (T2D) diabetes to overt DN and end-stage renal disease (ESRD) [1].

Diabetic nephropathy affects individuals with both T1D and T2D with a prevalence ranging from 25 to 40 %, even when glucose control is nearly optimal [1, 2], and 44 % of new cases of ESRD in the U.S. are caused by DN [3]. While strict glycemic control delays the onset of DN and is considered the first line of intervention by the American Diabetes Association guidelines to prevent diabetic complications [4], it cannot completely prevent the progression of DN. To date, management of DN is based on controlling blood glucose level blood pressure and associated risk factors, on preserving systolic/diastolic function and on reducing proteinuria with the use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers [5–9]. However, recent clinical trials failed to halt the progression to ESRD, and the overall success rate of new therapeutics to entry into the clinical setting is extremely poor [7]. Because of the aforementioned issues, new therapeutic strategies are needed to improve and eventually restore regular kidney function in individuals with DN.

Recent studies have documented a significant role for inflammation in the pathogenesis of DN [10–12]. A role for cells of the innate (i.e., macrophages) and adaptive immune systems (i.e., T cells) and of cytokine/chemokine release has also been recently discovered [11, 13]. However, strategies based on the targeting of a single pathway or even of a network of the inflammatory cascade failed to prevent the progression to ESRD [7, 14]. It is possible that the presence of already established fibrosis as well as our inability to detect early and non-invasively alterations in kidney metabolism and morphology may limit the effect of novel therapies [11, 15, 16]. An increase in extracellular matrix deposition in the basal glomerulus, in the tubular membrane, in the mesangium and in the interstitium is evident in DN [2]. Immunosuppressive and anti-inflammatory drugs (i.e., calcineurin inhibitors, nonsteroidal anti-inflammatory drugs) do not represent the solution to reduce the contribution of inflammation into DN progression, due to the fact that they may worsen kidney function [17, 18].

Stem cells (SCs) are characterized by several unique features, including pluripotency and self-renewal, and SCs are immunoprivileged cells with the ability to regenerate virtually all adult tissues and organs [19–22]. SCs can be isolated from several sources such as embryos, cord blood, bone marrow and several adult tissues or organs, including the kidney [19, 23]. Their recently confirmed immunoregulatory and reprogramming properties suggest that SCs can modulate the immune response and exert anti-inflammatory functions [19]. In addition, growing evidence suggests that replenishing and regulating the organ stem cell niche represents a key mechanism to preserve homeostasis and function [24], thus supporting the use of SCs as a therapeutic tool in kidney diseases. In this review, we will examine in depth how immunological and anti-inflammatory properties of SCs can be harnessed to establish a novel therapeutic option for DN.

Stem cells in the treatment of diabetes

The use of stem cells in the treatment of diabetes, particularly of type 1 diabetes, is an important topic that may actually influence diabetic complications as well. A stem cell-based treatment of diabetes might prevent the development of late complications, such as diabetic nephropathy and the following end-stage renal disease. The rationale for the use of SCs in T1D is based upon their immunoregulatory properties, which may help to rescue peripheral tolerance toward pancreatic β cells by reshaping the immune response and blocking their assault by autoreactive T cells [21]. In preclinical models, bone marrow mesenchymal SCs (BM-MSCs) have been shown to cure newly diabetic non-obese diabetic (NOD) mice by taking advantage of a hypoimmunogenic phenotype and a broad range of immunomodulatory capabilities [22]. Also, cord blood SCs reverted hyperglycemia in NOD mice by facilitating the generation of regulatory T cells, thus controlling autoimmune response [21]. Hematopoietic SCs (HSCs) have been recently tested in humans as a novel therapeutic strategy to treat T1D by rescuing peripheral tolerance toward pancreatic β cells and showed encouraging results [25]. In this regard, a SC-mediated remission of T1D may prevent/delay the development of long-term T1D complications, including DN. Few data are available on the use of SCs in T2D, in which the inflammatory component has been revealed only recently.

The case of inflammation in diabetic nephropathy

Immune cells

Several recent lines of evidence implicate inflammation as a potential pathogenic mechanism for the development and progression of DN [13, 26, 27]. In the early stage of DN, T cells and macrophages migrate and accumulate in glomeruli and interstitium, due to the local release of adhesion molecules and chemokines [27]. The process is initiated by cells infiltrating the kidney, which release proinflammatory cytokines (IFN-γ, TNF-α and IL-1β) and reactive oxygen species (ROS), thus triggering stress-activated protein kinases, p38 MAPK, and JNK signaling pathways [28]. Renal cells subsequently react by releasing chemokines (MCP-1 and CSF-1) and profibrotic factors such as TGF-β [15], thus establishing an inflammatory loop [12] which favors the deposition of extracellular matrix components including type I, II, IV collagen and fibronectin [29–31].

Interestingly, recent evidence suggests that renal cells may ectopically express immune-related molecules. For instance, podocytes potentially express costimulatory molecules during hyperglycemia in vivo (i.e., B7-1), as this has been shown in other diseases (e.g., focal segmental glomerulosclerosis) [32], thus triggering T-cell activation and maintaining inflammation [33].

Cytokines/chemokines

Proinflammatory cytokines such as IFN-γ, TNF-α, IL-1β and IL-6, released primarily by leukocytes infiltrating the kidney during DN, are thought to initiate and maintain an inflammatory environment, thus favoring the progression toward fibrosis [11]. Blockade of cytokine and chemokine action in preclinical studies resulted in the attenuation of kidney hypertrophy and of deposition of extracellular matrix [34] in the mesangium, podocytes, interstitium and proximal tubule, thus confirming the important role of cytokines and chemokines in DN [29–31]. Interestingly, Lim et al. reported that blocking c-fms, a receptor for CSF-1 (one of the major cytokines involved in macrophage accumulation) in a mouse model of early stage DN, abrogated inflammation and prevented the progression of DN [35]. Other anti-inflammatory strategies (rapamycin, pentoxifylline and COX inhibitors) have successfully delayed the progression of DN in murine models; however, none of the aforementioned approaches was capable of preventing/reverting the onset of the disease [11]. Interestingly, Abatacept, which abrogates B7-1 signaling in podocytes, prevents functional and morphological features of DN in db/db mice [33]. Finally, renal cells may produce different chemokines as well, such as RANTES (CCL5), interleukin-8 (CXCL8), IFN-γ inducible protein (CXCL10) and monocyte-chemoattractant protein-1 (MCP-1) in an inflammatory environment [28]. The blockade of CCR2 (the receptor for MCP-1) was also shown to reduce glomerular sclerosis in a murine model of DN [36].

Transcription factors and adhesion molecules

Experimental evidence has highlighted the ability of p38 mitogen-activated protein kinase (MAPK) to promote the induction of a proinflammatory environment in the kidney [28]. Clinical studies have shown that p38 MAPK activity is upregulated in DN and that it is associated with the development of the disease [37]. Lim et al. [28] demonstrated that p38 MAPK signaling inhibition in obese diabetic mice reduced MCP-1 levels and macrophage infiltration, improved albuminuria and preserved podocytes. Another transcription factor activated by hyperglycemia is nuclear factor-κB (NF-κB) [38], which binds the promoter regions of genes encoding chemokines (such as MCP-1) and adhesion molecules (such as ICAM-1), known to be major players of inflammation and ECM deposition. Blocking NF-κB with curcumin significantly improved diabetic renal injury by reducing macrophage infiltration and the release of proinflammatory chemokines [39]. Genetic deficiency of adhesion molecules such as ICAM-1 rendered db/db mice resistant to DN and protected them from the development of the disease [40]. Finally, toll-like receptor 4 (TLR4), which activates different proinflammatory transcription factors (NF-κB, MyD88 and CCL), is increased in human renal tubules obtained from kidney biopsies of DN patients [41].

Embryonic stem cells

Despite the lack of experimental evidence regarding the possible use of embryonic stem cells (ESCs) in DN, ESCs appear to be immunoprivileged and possess immunoregulatory properties when examined in alloimmune settings [42, 43]. Firstly, ESCs express low levels of MHC-I and do not express MHC-II surface molecules [19]. Secondly, while ESCs upregulate the expression of MHC-I—but not MHC-II—when differentiated [42], they were shown to retain their immunoregulatory properties and induce T-cell apoptosis via FasL [44]. Murine ESCs have been shown to abrogate the in vitro alloimmune response and inhibit T-cell proliferation via a cell–cell contact mechanism [43, 45]. However, development of proliferative abnormalities, including teratoma and teratocarcinomas, may still represent an important issue in the use of ESCs in the clinical setting [46, 47]. Adequate cell isolation techniques may ensure progenitor purity, thereby overcoming or reducing the possibility of teratoma formation [47]. The presence of immunogenicity, despite being weak, necessitates some sort of immunosuppressive regimen if ESCs are to be used in the clinical setting, unless investigators can succeed in HLA engineering of ESC lines by either deriving HLA-homozygous subclones from HLA-heterozygous ESC lines or by generating HLA-negative ESCs [48, 49]. This may allow for investigation and initiation of a novel therapy, in which ESCs are used for their immunoregulatory and regenerative properties, without any additional treatment, such as in therapy for DN.

Cord blood stem cells

The experimental evidence on the potential use of cord blood stem cells (CB-SCs) in renal diseases has been documented quite recently. In 2010, Morigi et al. [50] demonstrated strong improvement in renal function and a marked reduction in renal tubular damage following

intravenous infusion of human cord blood mesenchymal stem cells (hCB-MSCs) in a cisplatin-induced murine model of acute kidney injury. In 2011, Chung and colleagues evaluated the effect of intravenously infused hCB-MSCs in a mouse model of lupus nephritis [51]. Interestingly, hCB-MSC transplantation not only significantly reduced proteinuria but also promoted the transition from a proinflammatory to an anti-inflammatory environment (by decreasing IFN-γ, IL-2, TNF-α, IL-6 and IL-12 levels and by increasing IL-4 and IL-10 levels) [51]. Investigators suggested that improvement in renal function in hCB-MSC-treated mice was due to an immunomodulatory effect, rather than through local engraftment and differentiation into renal cells [51]. With direct relevance to DN, the effect of the administration of hCB mononuclear cells (CB-MNCs) in a murine model of streptozotocin-induced DN has been studied [52]. While in control mice there was an increase in serum creatinine, in blood urea, in albumin/creatinine ratio and in renal laminin, suggesting global dysregulation of kidney morphology and function [52], the infusion of CB-MNCs resulted in a significant improvement in all compromised parameters, including a reduction in tubular dilatation and glomerular hypertrophy [52]. This body of evidence supports the potential use of CB-SCs in DN, particularly because of their safety and relative ease of collection [19, 53]. Moreover, the drawback of the limited number of cells per cord blood unit can be overcome with the use of expansion techniques [19].

Adult stem cells

Adult stem cells reside in immunoprivileged protective microenvironments called stem cell niches, within specific organs and tissues (intestine, heart, bone marrow, kidney and brain) and serve to preserve local homeostasis and function [24]. Usually located at the urinary pole of the Bowman's capsule in adult kidneys, renal progenitor cells are involved in the turnover of resident renal epithelial cells, thus maintaining the balance between injury and regeneration [54]. However, several studies have emphasized a critical role for bone marrow mesenchymal stem cells (BM-MSCs) and bone marrow hematopoietic stem cells (HSCs) in promoting repair and regeneration of renal structures after injury because of their capacity to be recruited to inflamed/injured areas by local release of chemokines [54, 55]. Consistent with this, injection of autologous mesenchymal or hematopoietic adult stem cells

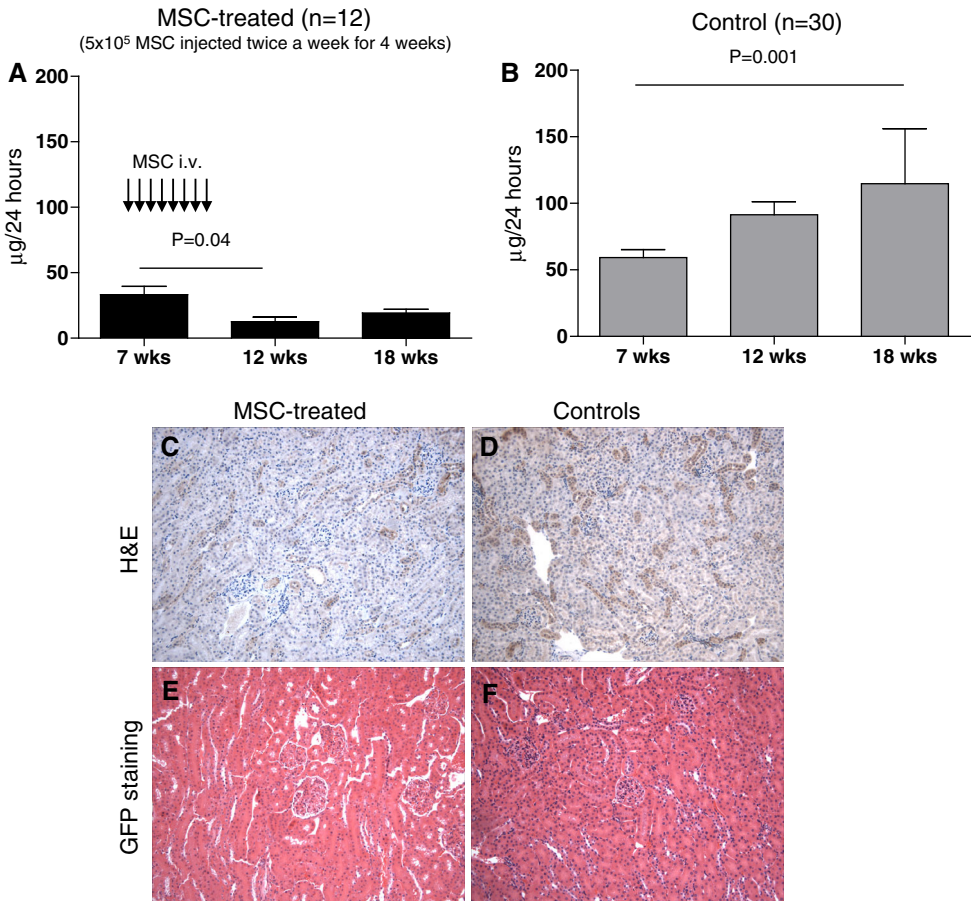

Fig. 1 Improvement in urinary albumin excretion following allogeneic infusion of BM-MSCs in db/db DN mice (**a**) compared with control untreated mice (**b**). H&E staining showed a diffuse-reduced kidney infiltration, with preserved kidney morphology in MSC-treated db/db mcie (**c**) compared with untreated controls (**d**). GFP-labeled BM-MSCs were undetectable in the kidney of db/db DN mice one month after injections (**e**, **f**). *BM-MSCs* bone marrow mesenchymal stem cells, *DN* diabetic nephropathy, *H&E* hematoxylin and eosin staining, *GFP* green fluorescent protein. (Fiorina et al., unpublished data)

may promote renal repair via two non-mutually exclusive mechanisms: (i) blocking local immune/inflammatory responses and halting renal injury and (ii) stimulating differentiation and proliferation of renal progenitor cells [55–57]. A third proposed mechanism posits direct differentiation of BM-SCs into renal cells but is losing favor. In an experimental study performed by Ezquer et al. [58], BM-MSCs were infused into an STZ-induced diabetic murine model, which resulted in a reduction in albuminuria, in glomerular hyalinosis and in mesangial expansion after two months following treatment [58]. Zhou et al. [59] demonstrated that intracardiac infusion of BM-MSCs in a rat model of diabetes improved DN, reduced blood glucose levels and improved urine albumin/creatinine ratio and kidney enlargement. BM-MSCs were located primarily in renal interstices rather than in renal tubules or in glomeruli, suggesting that the effects exerted by BM-MSCs were a consequence of a paracrine mechanism initiated by the transplanted cells, rather than the direct differentiation of BM-MSCs into renal cells [59]. In a recent experimental study conducted by Wang et al. [60], infusion of BM-MSCs via the left renal artery was able to prevent kidney injury and to reduce podocyte loss in STZ-treated rats. Sixty days after local BM-MSC infusion into the left kidney, significant reduction in mesangial matrix deposition as well as decreased loss of podocytes was observed in the left kidney of treated animals when compared to the left and right kidneys of untreated DN rats and to the right kidney of STZ-treated DN rats [60]. Interestingly, high levels of BMP-7 (a survival factor for podocytes) were observed in the left kidney of treated animals, suggesting again that the protective effect exerted by BM-MSCs was paracrine [60]. Investigators also have reported that BM-MSC infusion reduces albuminuria and restores glomerular nephrin and podocin expression [60]. Our group showed that the infusion of allogeneic BM-MSCs abrogates the increase in urinary albuminuria in db/db mice, but transplanted cells were not detectable in the kidney 1 month after infusion (Fiorina P et al., unpublished data) (Fig. 1). BM-MSCs have been shown to exert an immunosuppressive effect by inhibiting the proliferation and function of T, B and NK cells [61–63] via a cell/cell contact mechanism mediated by the PD-1/PD-L1/PD-L2 pathway, which has important immunoregulatory functions [64, 65], and also by the release of humoral factors, such as TGF-β1 or prostaglandin E2 (PGE-2) [66]. Recently, the administration of autologous adipose-derived mesenchymal stem cells (AD-MSCs) was able to ameliorate DN in STZ-induced diabetic rats [67]. Investigators found that AD-MSCs migrate to the renal parenchyma, restore the widening of the mesangium and reduce oxidative stress, resulting in an overall renoprotective effect [67]. This effect was determined to be a consequence of significant inhibition of proinflammatory cytokines (IL-6, IL-1β and TNF-α) promoted by AD-MSCs [67]. Hence, harnessing the immunological and pluripotent abilities of autologous adult stem cells, particularly of BM-SCs, may represent a novel potential approach to treat DN [21].

Induced pluripotent stem cells

In a mouse model of acute kidney injury, murine-induced pluripotent stem cells (iPS) reduced the expression of proinflammatory cytokines, thus favoring the creation of an anti-inflammatory environment [68]. However, immunological properties of human iPS are not yet well characterized. iPS, as embryonic stem cells, express low levels of surface HLA class I-related molecules but do not express HLA class II molecules [69]. The prospect of obtaining patient-specific pluripotent stem cells from somatic cells is, without doubt, of high interest in the field of regenerative medicine [70]. The low efficacy of reprogramming and high costs related to the procedure [71], the risk of

Table 1 Preclinical findings confirming the potential use of stem cells as treatment for diabetic nephropathy

Stem cell source	Preclinical evidence	References
CB-SCs	CB-SCs improve glomerular function in a murine model of DN	Masoad et al. [52]
BM-MSCs	BM-MSCs reduce glycosuria, albuminuria and mesangial expansion in a murine model of DN	Ezquer et el. [58]
BM-MSCs	BM-MSCs infused in a murine model of DN improve albumin/creatinine ratio and kidney enlargement through a paracrine mechanism	Zhou et al. [59]
BM-MSCs	BM-MSCs prevent the development of albuminuria in a rat model of DN and restore nephrin and podocin expression	Wang et al. [60]
ESCs	ESCs abrogate the alloimmune response and inhibit T-cell proliferation in vitro	Drukker et al. [43] Fandrich [45]
CB-MSCs	CB-MSCs reduce proteinuria in a murine model of lupus nephritis, creating an anti-inflammatory environment	Chang et al. [51]
AD-MSCs	AD-MSCs reduce glomerular mesangial widening and oxidative stress in a murine model of DN	Fang et al. [67]
iPS	Murine iPS promote an anti-inflammatory environment and reduce the expression of proinflammatory cytokines	Lee et al. [68]

CB-SCs cord blood stem cells, *DN* diabetic nephropathy, *BM-MSCs* bone marrow mesenchymal stem cells, *ESCs* embryonic stem cells, *AD-MSCs* adipose-derived mesenchymal stem cells, *iPS* induced pluripotent stem cells

teratoma formation following undifferentiated iPS transplantation [72] and the potential onset of genetic abnormalities in cells derived form iPS differentiation [73] are the major concerns related to iPS that should be resolved before moving to the clinical setting.

Lessons learned from ongoing clinical trials in other kidney diseases

The enhanced characterization of the immunological and regenerative potential of stem cells, acquired through years of experimental research (Table 1), has led to the initiation of numerous and currently ongoing clinical studies, in which the therapeutic effect of stem cells is evaluated in the context of kidney disease (Table 2). Liang et al. [74] reported that allogeneic infusion of BM-MSCs in patients with persistently active systemic lupus erythematosus was able to ameliorate the clinical condition as well as improve renal function. In particular, at 1 year of follow-up, a reduction in proteinuria was observed in 11 out of 13 patients, while the remaining two patients relapsed [74]. In addition, in two patients, an improvement in glomerular filtration rate was detected [74]. With regard to DN, currently there is only one active clinical trial. In this study, which is ongoing in Melbourne (Australia), the safety and efficacy of a single intravenous infusion of allogeneic mesenchymal precursor cells (MPCs, Mesoblast) in adult patients with DN and T2D will be determined (ClinicalTrials.gov identifier: NCT01843387). The growing knowledge of stem cell biology will expand the field to increase the number of stem cell-based clinical trials in individuals with DN.

Conclusion

The development of novel therapeutic approaches represents an important goal to improve, if not to completely cure, the health of patients with diabetes and with impaired renal function. Experimental results (Tables 1 and 2) suggest the urgent need for clinical trials in order to clarify the potential of stem cells as treatment for DN. The understanding that inflammation plays an important role in the initiation and advancement of DN and that SCs have anti-inflammatory and immunomodulatory properties may widen the field to generate a novel and improved therapy. Even though mechanisms of diabetic nephropathy might be slightly different in T1D and T2D, we are suggesting that SCs may be useful for both diseases because they target the common inflammatory pathway.

Table 2 Stem cell-based ongoing clinical trials in non-oncological kidney diseases

Disease	Cells	Type of infusion	Aim of the study	Clinicaltrial.gov Identifier
Acute kidney injury	BM-MSCs (ex vivo expanded)	Allogeneic	To determine the feasibility and safety of ex vivo expanded BM-MSC infusion	NCT01275612
Chronic renal failure	BM-MSCs CB-MSCs	Allogeneic	To evaluate the safety and efficacy of the treatment	NCT01876017
Diabetic nephropathy	Mesenchymal precursor cell	Allogeneic	To evaluate the safety and efficacy of the treatment	NCT01843387
Kidney failure Liver failure	BM-MSCs	Allogeneic	To evaluate the safety and efficacy of the treatment	NCT01429038
Lupus nephritis	CB-MSC	Allogeneic	To evaluate the safety and efficacy of the treatment	NCT01539902
Occlusive kidney disease	AD-MSCs	Autologous	To evaluate the safety and toxicity of intra arterial infusion of AD-MSCs	NCT01840540
Renal failure	AD-MSCs	Autologous	To evaluate the safety and efficacy of the treatment	NCT01453816
Chronic allograft nephropathy	BM-MSCs (ex vivo expanded)	Autologous	To test if BM-MSCs can prevent organ rejection and maintain kidney function	NCT00659620
Chronic renal failure	BM-MSCs	Autologous	To evaluate the safety and efficacy of the treatment	NCT01152411
Lupus nephritis	BM-MSCs	Autologous	To evaluate the safety and efficacy of the treatment	NCT00659217

BM-MSCs bone marrow mesenchymal stem cells, *CB-SCs* cord blood stem cells, *AD-MSCs* adipose-derived mesenchymal stem cells

Acknowledgments Paolo Fiorina is the recipient of a JDRF Career Development Award, an ASN Career Development Award and an ADA Mentor-based Fellowship grant. P.F. is also supported by a Translational Research Program (TRP) grant from Boston Children's Hospital, a Harvard Stem Cell Institute grant ("Diabetes Program" DP-0123-12-00) and an Italian Ministry of Health grant (RF-2010-2303119, RF-2010-2314794 and RF-FSR-2008-1213704). Francesca D'Addio is the recipient of an Italian Scientists and Scholars of North America Foundation (ISSNAF)-Fondazione Marche Fellowship.

Conflict of interest Paolo Fiorina discloses Sorgente S.r.l stock ownership and Otsuka grant funding. Francesca D'Addio, Alessio Trevisani, Moufida Ben Nasr, Roberto Bassi, Basset El Essawy, Reza Abdi, Antonio Secchi declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain any studies with human subjects performed by the any of the authors. Animal studies were conducted in accordance with institutional and National Institutes of Health guidelines and Institutional Animal Care and Use Committee (IACUC) approval.

Statement of informed consent We would like to mention that there are no patients in this study.

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