Mesenchymal stromal cell-based therapy in kidney diseases and transplantation

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ABSTRACT

Intense investigation in pre-clinical models of kidney disease and transplantation showed that mesenchymal stromal cell (MSC) therapy acts on renal and inflammatory cells in multiple, complex and integrated ways, resulting in cell repair and regeneration, in the inhibition of inflammatory cells, and in the development of cells endowed with their own anti-inflammatory and immune-regulatory properties. These encouraging data paved the way for exploring the use of MSC in clinics as innovative therapeutic tools for patients with renal diseases and transplantation. In this review, we describe the available results of clinical studies of MSC in patients with post-cardiac surgery, acute kidney injury, chronic kidney diseases - including diabetes, renovascular disease and lupus nephritis - and in kidney transplant recipients, with a particular focus on our experience with MSC therapy as a pro-tolerogenic strategy in kidney transplantation. The available studies, mainly phase 1, indicated that MSC therapy is safe and feasible and not associated with adverse events, at least in the short- and mid-term. Encouraging results have been reported in renovascular disease and kidney transplantation, while studies in acute kidney injury and chronic kidney disease had contrasting outcomes. The relevant issues and the knowledge gap that still limit the translation of MSC cell therapy into clinical practice are discussed briefly.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSC) are a heterogeneous population of non-hematopoietic cells obtained after ex vivo expansion of adherent cells from bone marrow, adipose tissue, umbilical cord and blood and other tissues. Despite the intense debate in the scientific community about their nature, in vivo counterpart and definition, MSC - irrespective of their tissue origin - are still defined by the minimal criteria established 10 years ago by the International Society for Cellular Therapy (ISCT), i.e.: plastic adherence under standard culture conditions, expression of CD105, CD73 and CD90 molecules, being negative for the expression of CD45, CD34, CD19 and CD79 molecules, and trilineage (chondrocyte, adipocyte and osteoblast) differentiation in vitro in the presence of proper growth factors. Ex vivo expanded MSC product displays unique pro-regenerative, reparative, anti-inflammatory and immune-regulatory properties that have stimulated the development of MSC as innovative therapy for inflammatory and immune-mediated diseases. The ease with which they expand and their immune-evasive phenotype have encouraged small and medium-sized businesses and big pharmaceutical companies to invest in the commercial and large-scale production of well-characterized, high-quality, low-cost MSC preparations readily available for clinical use.

Over the past two decades, intense investigation into kidney disease and transplant models has shown that MSC perform multiple, integrated and complex actions targeting every single renal cell, eventually resulting in the activation of reparative and pro-regenerative programs in tubular and glomerular cells, in the inhibition of effector functions of inflammatory cells and in the conversion of immune cells, such as macrophages, dendritic and T cells into cells endowed with unique pro-regenerative, reparative, anti-inflammatory and immune-regulatory properties. These encouraging data paved the way for exploring the use of MSC in clinics as innovative therapeutic tools for patients with renal diseases and transplantation. In this review, we describe the available results of clinical studies of MSC in patients with post-cardiac surgery, acute kidney injury, chronic kidney diseases - including diabetes, renovascular disease and lupus nephritis - and in kidney transplant recipients, with a particular focus on our experience with MSC therapy as a pro-tolerogenic strategy in kidney transplantation. The available studies, mainly phase 1, indicated that MSC therapy is safe and feasible and not associated with adverse events, at least in the short- and mid-term. Encouraging results have been reported in renovascular disease and kidney transplantation, while studies in acute kidney injury and chronic kidney disease had contrasting outcomes. The relevant issues and the knowledge gap that still limit the translation of MSC cell therapy into clinical practice are discussed briefly.

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Contributions: FC researched the data and wrote the article; GR made substantial contributions to content discussions and reviewed the manuscript.

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with their own immune-regulatory functions.\(^{45-51}\) (Figure 1). These encouraging pre-clinical data paved the way for exploring, in clinics, the use of MSC as an innovative therapeutic tool for patients with renal diseases and transplants.

In this review we describe the results of the clinical experiences with MSC of patients with acute kidney injury, chronic kidney diseases and kidney transplantation, with a particular focus on our experience with MSC therapy as a pro-tolerogenic strategy in kidney transplantation.

**Acute kidney injury**

Acute kidney injury (AKI) is a frequent and essentially treatment-resistant complication that is caused by different insults such as ischemia-reperfusion, nephrotoxins and sepsis.\(^{52-55}\) It is characterized by acute tubular cell death, damage to endothelial cells and microvessels, and by intense inflammation. AKI has a high morbidity and mortality rate and only conservative treatments are available.\(^{56}\) Moreover, even if patients survive their acute illness, the incompletely resolved inflammatory and fibrotic processes lead, in a significant proportion of patients, to chronic renal failure.\(^{54,57}\)

Based on encouraging preclinical data (Figure 1), phase 1/2 clinical studies in patients with AKI have been undertaken\(^{58-60}\) (Table 1\(^{58-74}\)), using the commercial allogeneic bone marrow (BM)-MSC AC607 product (Allocure). In a safety clinical study, 16 patients undergoing cardiac surgery and at particularly high risk of post-operative AKI received dose-escalating BM-MSC treatment at the end of surgery to prevent AKI development. The data indicate that MSC infusion was safe and feasible and the length of the hospital stay and readmission rates were reduced in MSC-treated individuals compared to historical case controls. In all MSC-treated patients, post-operative renal function remained stable and did not deteriorate during the 16-month follow-up in patients with underlying chronic kidney disease (CKD).\(^{58,59}\)

These positive clinical data have, however, been challenged by the very recently published results of a randomized, double-blind, placebo-controlled study of AC607-MSC in patients with established post-cardiac surgical AKI\(^{60}\) (Table 1). The study randomized 156 adult subjects with laboratory evidence of post-operative AKI (rise of 0.5 mg/dL in serum creatinine during the 48 h following removal of cardiopulmonary bypass) to receive intra-aortic MSC or placebo infusion. After 75% patient accrual, the trial was halted for futility, because no difference in time to kidney recovery, rate of dialysis or 30-day mortality was found between patients given MSC or placebo.\(^{60}\)

This study argues against the real utility of MSC therapy in clinical AKI. However, it should be considered that, compared to the prior explorative clinical study and studies in pre-clinical models, in this study better designed with appropriate sample size MSC were however infused when serum creatinine had already increased considerably. This approach could have entailed a delayed diagnosis of AKI, since it is known that creatinine accumulates in the serum 1-2 days after the onset of AKI, and MSC were consequently administered in a context of overwhelming injury.

Thus, it remains to be established whether MSC are more effective for preventing than treating AKI, and whether earlier intervention - guided by more sensitive biomarkers such as Kim-1 and NGAL,\(^{61-63}\) whose levels increase early during the initiation phase of AKI - can still limit ongoing injury.

We designed a clinical trial to evaluate the safety and feasibility of systemic infusion of allogeneic BM-MSC in patients with solid organ cancers who develop AKI after chemotherapy with cisplatin. In this study, escalating doses of BM-MSC would have been i.v. infused in patients showing evidence of AKI, defined as a >3500% increase over baseline values of urinary NGAL concentrations at day 2 post-cisplatin infusion (NCT01275612). However, despite extensive screening, none of the seventeen screened patients met this primary criterion of acute renal failure development and the study was recently halted.

**Chronic kidney disease**

Chronic kidney disease is a syndrome of progressive deterioration of kidney function over time with significant implications for patients’ health.\(^{64}\) Nowadays, CKD has become a public health priority, as highlighted by the Global Burden of Diseases (GBD) 2016 study, which ranked CKD as the nineteenth cause of mortality, compared to its 1990 ranking, when CKD was ranked the twenty-seventh cause of mortality. Despite renoprotective therapies, such as blockade of the renin-angiotensin system, a consistent proportion of CKD patients progress to end-stage renal failure, requiring renal replacement therapy.\(^{64,65}\)

So far, two clinical studies have evaluated the effects of autologous BM-MSC\(^{66}\) or extracellular vesicles derived from umbilical cord (UC)-MSC\(^{67}\) in patients with CKD (Table 1). In a single-center study, seven patients with non-diabetic CKD were given an intravenous infusion of autologous BM-MSC.\(^{66}\) The infusion was safe and no adverse events were observed. The patients were followed-up until 18 months post-cell infusion and clinical parameters recorded during this period were compared to those recorded from the 18 months prior to enrolment to cell infusion. No significant differences in serum creatinine levels and estimated glomerular filtration rate (eGFR) were found between the two study periods, ruling out the possibility that...
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MSC had a significant effect on renal function. However, the low number of patients with very heterogeneous causes of CKD, as well as the advanced stage of most patients included in this study (four out of the seven patients developed end-stage renal disease requiring renal replacement therapy during or immediately after the 18-month follow-up), do not make any generalized conclusions about the efficacy of BM-MSC therapy in CKD possible.

In a single-center, randomized, placebo-controlled pilot study, 40 patients with CKD were randomized to receive placebo or two doses of extracellular vesicles (EV) isolated from UC-MSC (Table 1). The procedure was safe. Patients given MSC-EV showed an increase in eGFR and a reduction in blood urea and creatinine levels during the 12-month follow-up, not observed in the placebo group, suggesting a trend towards transient improvement in kidney function following EV injections. These findings should be confirmed in future clinical trials using, however, well...
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<td>Single intra-aortic injection, dose-escalating (doses not specified), of commercial BM-derived MSC (Allocure)</td>
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<td>Single intra-aortic injection of 2×10⁶/kg of commercial BM-derived MSC (Allocure)</td>
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<td>An intravenous injection followed by a second intra-arterial injection (7 days apart) of 100 µg/kg/dose of UC-MSC EV (corresponding to 1×10⁶ MSC/kg/dose)</td>
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**Chronic kidney disease**

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MSC, mesenchymal stromal cells; BM, bone marrow; AKI, acute kidney injury; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; EV, extracellular vesicles; SLE, systemic lupus erythematosus; AT, adipose tissue; UC, umbilical cord; MPC, mesenchymal precursor cells; MMF, mycophenolate mofetil; WHO, World Health Organization.
characterized EV preparation that fit better with the recommendations of the International Society of Extracellular Vesicles (ISEV) regarding the quality and characterization of the cell product.

Renovascular disease

In an open-label, single-center, dose-escalation trial, 14 patients with renovascular disease received an intra-arterial injection of autologous adipose-tissue derived MSC in the stenotic kidney on top of standardized medical treatment (Table 1). Control patients matched for age, kidney function and severity of stenosis received only the medical treatment. MSC treatment was well-tolerated and was associated with a significant increase in renal cortical blood flow, perfusion and renal oxygenation, both in the stenotic and the contralateral kidney, whereas no changes were observed in control patients. In MSC-treated patients, GFR measured by iothalamate clearance was preserved, compared to control patients in whom GFR underwent a significant decline.

The positive findings of this study underline MSC as a promising therapeutic application for ameliorating vascular insufficiency and inflammatory injury in ischemic kidney disease.

Diabetic kidney disease

In a randomized, double-blind, dose-escalating placebo-controlled study, type 2 diabetes (T2D) patients with moderate to severe diabetic kidney disease (DKD) were randomized to receive placebo or intra-venous injections of an escalating dose of 150×10⁶ or 300×10⁶ mesenchymal precursor cell (rexlemestrocel-L) (Table 1). Cell infusion was well tolerated and no adverse events were observed during the 60-week follow-up. As the explorative efficacy endpoint, estimated and measured GFR changes from baseline to 12 weeks post-treatment were evaluated, and results indicated a trend towards stabilized or improved estimated and measured GFR in patients given rexlemestrocel-L, irrespective of the dose. These data, demonstrating the safety and feasibility of the procedure, should be confirmed in larger and powered clinical trials, possibly aimed at evaluating whether multiple MSC infusions can foster the minimal beneficial effect on renal function observed in this study.

We are coordinating a multicenter European study (the Novel Stromal Cell Therapy for Diabetic Kidney Disease, the NEPHSTROM study, NCT02585622) with a standardized cell product (the allogeneic BM-MSC preparation ORBCEL-M, Orhsen Therapeutics) in T2D patients with progressive DKD and mild to moderate renal insufficiency. Patients are randomized to placebo or to an escalating dose of MSC (80×10⁶, 160×10⁶, and 240×10⁶). The primary outcome is safety and feasibility of ORBCEL-M administration, with a pilot explorative efficacy outcome on the effect of the cellular therapy on a measured GFR decline over a study period of 18 months.

Lupus nephritis

MSC therapy in patients with systemic lupus erythematosus (SLE) has been conducted in China, mainly by the Sun et al. group,71-73 with contrasting results (Table 1).

Starting from a preliminary observation of clinical improvement in 4 SLE patients given i.v. infusion of allogeneic BM-MSC, overall more than 80 patients with severe and drug-refractory SLE were treated with BM- or UC-MSC by the Sun group.72,73 In a report focusing specifically on the 12-month follow-up of 81 patients with lupus nephritis unresponsive to conventional immunosuppressive treatment,72 the authors found that either BM- or UC-MSC treatment induced partial or complete renal remission in 60% of patients. Total SLE disease activity index (SLEDAI) and British Isles Lupus Assessment Group (BILAG) renal activity scores declined significantly compared to baseline values; proteinuria also decreased, and GFR improved. However, during the 12-month follow-up, 24% of patients who had previously achieved renal remission experienced relapse. A significant correlation between remission and baseline proteinuria and creatinine levels was found, suggesting that baseline renal disease severity can influence the response to cell therapy.72 The recent paper on the long-term follow-up of the overall population of lupus patients given MSC cell therapy at this center reports a 5-year overall survival rate of 84%, complete or partial clinical remission in 27% and 7% of patients, respectively, and an overall relapse rate of 24%.73 Overall, this clinical experience suggests that MSC could be a real therapeutic option for SLE patients refractory to conventional treatments. However, these studies lacked a control group and well-designed and placebo-controlled clinical trials are needed to demonstrate MSC efficacy in SLE.

Along similar lines, a randomized, double-blind, placebo-controlled trial recently assessed the effect of UC-MSC in patients with severe lupus nephritis (Table 1). Eighteen patients were randomized in a 2:1 ratio to receive double i.v. infusions of UC-MSC or placebo. All patients received induction therapy with intravenous methylprednisolone and cyclophosphamide and maintenance immunosuppression with oral prednisolone and mycophenolate mofetil (MMF). The results showed that a similar proportion of patients in the UC-MSC and placebo groups achieved remission. SLEDAI and BILAG scores and renal function improved in both groups at a comparable level. The trial was discontinued after the enrolment...
of the first 18 patients, when the investigators considered that the trial had failed to demonstrate any additional beneficial effects of UC-MSC therapy over standard immunosuppression. This study did not confirm previous, encouraging pilot studies in patients with SLE. However, it should be taken into account that the study by Deng et al. enrolled patients with newly diagnosed lupus nephritis who were given induction immunosuppressive treatment including cyclophosphamide and MMF, drugs known to be effective in inducing remission of lupus nephritis in the short-term. Indeed, five out of six patients in the control arm underwent remission, to the point that additional benefits from MSC on top of the already effective induction therapy could not be demonstrated.

Overall, no definitive conclusion can be drawn on the basis of current knowledge of lupus nephritis, even though MSC appear to be a promising second-line intervention for patients who are refractory to conventional immunosuppressive treatment and have moderate renal disease.

**Kidney transplantation**

Patients with kidney transplantation still receive life-long immunosuppression to prevent graft rejection, exposing them to a substantial risk of infections, malignancies and life-threatening drug side effects. Moreover, current immunosuppression performs poorly in preventing the development of chronic allograft nephropathy. Therefore, novel strategies to induce immunological tolerance and to reduce immunosuppression are needed.

In several pre-clinical models of organ transplantation, including skin, the heart, and kidney, the infusion of MSC resulted in significant graft survival prolongation. MSC mitigate the anti-graft T cell immune response, and, by inducing regulatory dendritic cells or regulatory T cells, also promote the induction and maintenance of immunological tolerance (reviewed in80). Several small pilot studies and a large clinical trial conducted in China assessed safety and feasibility and explored initial efficacy of autologous or third-party (from a subject unrelated to the organ donor and to the recipient) BM- or UC-MSC in kidney transplantation to alleviate chronic histological damage and subclinical rejection, to enable anti-rejection drug minimization, and to promote the development of immunological tolerance toward the graft (Table 2).

In a small study, autologous BM-MSC were infused twice in six living-donor kidney transplant recipients showing signs of subclinical rejection and/or increases in interstitial fibrosis/tubular atrophy (IF/TA) on their 6-month protocol biopsies (Table 2). Cell infusions were tolerated well and surveillance biopsies performed in two patients after MSC treatment showed tubulitis and IF/TA resolution. However, three patients developed an opportunistic viral infection, raising concerns regarding generalized immunosuppression after MSC treatment.

This concern was allayed by a large Chinese clinical trial that used MSC infusion to replace induction therapy with the anti CD25-antibody, basiliximab, in living-donor kidney transplant recipients (Table 2). Living-donor kidney transplant patients were randomized into 3 study groups: patients given autologous BM-MSC and either standard-dose (n=53) or 80% of the standard calcineurin-inhibitor (CNI) dose (n=52) or given induction therapy with basiliximab and standard CNI dose (n=51, control group). In this study, a significantly decreased risk of opportunistic infection was observed in MSC-treated patients compared to controls, alleviating concerns regarding possible global immunosuppression following MSC therapy in already immunocompromised kidney transplant recipients. Moreover, MSC-treated patients had faster renal function recovery during the first month and a significantly reduced incidence of acute rejection 6 months post-transplant, compared to the control group, in which an unexpectedly higher percentage of patients (22%) experienced acute rejection. The incidence of acute rejection at 1 year post-transplant was similar among patient groups. This study confirms that autologous BM-MSC infusion is safe and that MSC therapy can effectively replace basiliximab induction therapy and enable CNI dose reduction. Despite very encouraging results, this study did not attempt to evaluate the effect of MSC on recipient immune cells and also raised the question of whether costly MSC-based therapy should be used only as induction therapy to prevent acute rejection in kidney transplantation (a condition that is already controlled well by current low-cost immunosuppressive drugs).

A recent prospective multi-center study was performed using UC-MSC as induction therapy to prevent both delayed graft function (DGF) and acute rejection in deceased-donor kidney transplant recipients (Table 2). Forty-two renal allograft recipients receiving paired graft donation were divided into the control or trial group and given a double infusion of UC-MSC. MSC-treated patients tolerated the cell infusion with no adverse events. The incidence of DGF and acute rejection and the kidney graft function (by eGFR levels) were similar in patients given and not given MSC therapy. In addition, this paired study was performed in kidney transplant recipients of non-ECD kidneys (donor aged <65, no history of kidney disease, uncontrolled hypertension and diabetes), already at low risk of DGF development, thus making it difficult to detect any additional effect of MSC on DGF development.
Other investigators evaluated the possibility of using lower doses of maintenance immunosuppressive drugs after allogeneic BM-MSC cell therapy\(^8\)\(^2\) (Table 2). Donor-derived (even though it is not clear whether MSC were from the organ donor or from an unrelated BM donor) BM-MSC were infused in 16 living related donor kidney transplant patients. MSC infusion was combined with a 50% dose of tacrolimus, while the control group received the standard tacrolimus dose. Patients were followed-up for 24 months. Graft and

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<td>Two intravenous infusions of 0.1-1×10⁶/kg autologous BM-MSC, seven days apart, 6-10 months post-transplant</td>
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<td>Two intravenous infusions of 1-2×10⁶/kg autologous BM-MSC during surgery and 2 weeks later</td>
<td>Living-donor kidney transplant recipients n=53 combined with standard CNI dose n=52 combined with 80% CNI dose</td>
<td>Comparison with patients given paired kidneys, n=21 (1 year post-transplant)</td>
<td>Increased eGFR during the follow-up, lower incidence of acute rejection at 6 but not at 12 months, decreased risk of opportunistic infection</td>
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<tr>
<td>NCT02012153 (phase 1)(^7)(^8)</td>
<td>Single intravenous infusion of 1.7-2×10⁶/kg autologous BM-MSC, the day before transplant</td>
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<td>Comparison with control groups of living (n=3) or deceased (n=6) donor kidney transplant patients (1 year) (5-7 years)</td>
<td>Transient renal insufficiency following cell infusion Donor-specific CD8(^+) T cell unresponsiveness, high Treg/memory CD8(^+) T cells ratio; naïve and transitional B cell expansion (1 patient)</td>
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MSC, mesenchymal stromal cells; BM, bone marrow; CNI, calcineurin inhibitors; MMF, mycophenolate mofetil; IF/TA, interstitial fibrosis/tubular atrophy; eGFR, estimated glomerular filtration rate; UC, umbilical cord; DGF, delayed graft function; RATG, rabbit anti-thymocyte globulin; CsA, cyclosporine A.
patient survival, acute rejection, and graft function in the MSC-treated group did not differ from the control group, suggesting that MSC could enable safe minimization of maintenance immunotherapy.82

Overall, these studies demonstrated the safety and feasibility of the procedure and suggest MSC could have an immunomodulatory effect in kidney transplantation. However, most lack mechanistic studies to gain insights into the effect of MSC on the recipient anti-graft cell response.

In this regard, we are studying MSC therapy in kidney transplantation through a different approach.80-86

Based on experimental findings that the infusion of syngeneic BM-MSC induced cardiac graft survival prolongation in mice through the generation of donor-specific Tregs,49 we designed a pilot safety and feasibility study of autologous BM-MSC infusion in living-donor kidney transplant recipients.84 We first performed in vitro studies to evaluate the effect of immunosuppressive drugs on MSC immunosuppressive function and found that rabbit anti-thymocyte globulin (RATG), used in our protocol as induction therapy, bound to MSC, implying a possible detrimental effect of RATG on cell viability if MSC were infused during induction therapy. We therefore timed MSC infusion at the end of RATG administration (7 days post-transplantation), a point in time that also coincided with homeostatic proliferation of residual immune cells. This time frame would allow MSC to push peripheral Treg expansion, while constraining the proliferation of memory T cells. Therefore, we enrolled the first 2 patients given autologous BM-MSC at 7 days post-transplantation. Unexpectedly, a few days after MSC infusion both patients developed transient renal insufficiency. Histological analysis of a kidney graft biopsy performed in the second patient excluded on-fociness. Histological analysis of a kidney graft biopsy performed in the second patient excluded on-foceness. Histological analysis of a kidney graft biopsy performed in the second patient excluded on-foceness. Histological analysis of a kidney graft biopsy performed in the second patient excluded on-foceness. Histological analysis of a kidney graft biopsy performed in the second patient excluded on-foci

Conclusions

The available studies of MSC therapy in kidney disease and transplantation indicate the procedure is safe and feasible. To assess unwanted side effects, such as malignancies, MSC-treated patients should continue to be monitored long-term. Regarding efficacy, except promising results in kidney transplanta-
tion and renovascular disease, the impression is that clinical study outcomes in AKI and CKD have fallen short of expectations raised by encouraging pre-clinical animal data.

There are several issues that need to be considered: i) MSC employed in clinical studies are very different regarding the procedure for ex vivo expansion, tissue of origin, source (autologous or allogeneic) and whether they come from academic or commercial cell factories, so it is difficult to interpret and compare the data from these studies; ii) even if an identical MSC preparation is used to treat several patients, the treatment outcome will be affected by variables such as disease stage and severity and concomitant immunosuppressive drugs; iii) we have only a vague idea of how MSC interact with the host’s immune and non-immune cells.

Therefore, future research on MSC therapy should focus on better understanding the MSC mechanism of action, whether they really need to reach the diseased organ to act beneficially, or whether they exert a systemic hit-and-run effect through the release of soluble mediators and microparticles or by undergoing apoptosis, eventually educating the host immune cells themselves to mitigate tissue damage and inflammation. A better understanding of how and where MSC have beneficial effects will allow development of effective MSC preparation, will inform the rational selection of patients who will benefit from MSC therapy, and will guide the rational design of the best clinical trial strategy to fully exploit MSC’s potential. Questions regarding timing, whether to use multiple or single infusions, the minimum required dose, whether to use fresh or thawed MSC, autologous or allogeneic, and after minimal or extended in vitro expansion, are essential and need to be resolved definitively.

The field is still in its infancy and much work remains to be done. However, we believe that future research will move the clinical application of MSC-based therapy closer to being used for kidney diseases and transplantation.

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