

## Full Review

# Mesenchymal stromal cell therapy in conditions of renal ischaemia/reperfusion

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## ABSTRACT

Acute kidney injury (AKI) represents a worldwide public health issue of increasing incidence, with a significant morbidity. AKI treatment mostly relies on supportive manoeuvres in the absence of specific target-oriented therapy. The pathophysiology of AKI commonly involves ischaemia/reperfusion (I/R) events, which cause both immune and metabolic consequences in renal tissue. Similarly, at the time of kidney transplantation (KT), I/R is an unavoidable event which contributes to early graft dysfunction and enhanced graft immunogenicity. Mesenchymal stromal cells (MSCs) represent a heterogeneous population of adult, fibroblast-like multi-potent cells characterized by their ability to differentiate into tissues of mesodermal lineages. Because MSC have demonstrated immunomodulatory, anti-inflammatory and tissue repair properties, MSC administration at the time of I/R and/or at later times has been hypothesized to attenuate AKI severity and to accelerate the regeneration process. Furthermore, MSC in KT could help prevent both I/R injury and acute rejection, thereby increasing graft function and survival. In this review, summarizing the encouraging observations in animal models and in pilot clinical trials, we outline the benefit of MSC therapy in AKI and KT, and envisage their putative role in renal ischaemic conditioning.

**Keywords:** acute kidney injury, ischaemia/reperfusion, ischaemic conditioning, kidney transplantation, mesenchymal stromal cells

## INTRODUCTION

Acute kidney injury (AKI) is a broad clinical syndrome, currently defined as a rapid fall of glomerular filtration rate as reflected by an acute rise of serum creatinine and/or a decline in urine output [1]. The incidence of AKI is generally 5–7.5% in all acute care hospitalizations, but it accounts for up to 20% of admissions to intensive care units. Furthermore, ~30–40% of all cases of AKI during hospitalization are observed in operative settings, and particularly after cardiovascular surgery. AKI commonly involves tissue damage caused by transient ischaemia and reperfusion (I/R) [2]. The reduction or interruption of renal perfusion with a subsequent reflow induces significant cell metabolism perturbations and tissue inflammation. One of the paradigms of renal I/R is kidney transplantation (KT). Indeed, graft procurement, storage and transplantation requires the necessary temporary, and often predictable, interruption of renal blood flow. Still, such unavoidable I/R events contribute to early graft dysfunction and enhanced graft immunogenicity [3].

Treatment of AKI remains largely supportive, including fluid maintenance, vasoactive drugs, cytoprotective therapy and extra-renal epuration [4]. Recent advances in deciphering the pathophysiology of renal ischaemia/reperfusion injury (IRI) led to novel therapies, including non-invasive approaches of renal conditioning. Here, we discuss the putative role of mesenchymal stromal cell (MSC) therapy in AKI, as well as in KT.

## FEATURES AND PROPERTIES OF MSC

MSC represent a heterogeneous population of adult, fibroblast-like multi-potent cells characterized by their ability to differentiate into tissues of mesodermal lineages, including adipocytes, chondrocytes and osteocytes [5]. MSC can be isolated from various sources, including bone marrow, umbilical cord, adipose tissue or muscle [6]. According to the 2006 consensus of the International Society of Cellular Therapy, the distinctiveness of MSC relies on the following standard criteria: adherence to plastic surfaces; potential to differentiate into osteocytes, adipocytes and chondrocytes under standard *in vitro* differentiating conditions; surface expression of CD105, CD73 and CD90 and lack of the haematopoietic markers, CD45, CD34, CD14, CD11b, CD79a and HLA-DR.

A large number of *in vitro* and *in vivo* studies have documented the anti-inflammatory and immunoregulatory properties of MSC in both the innate and the adaptive immune systems. Indeed, MSC have been reported to prompt T-cell expansion towards a regulatory phenotype. These regulatory T cells (Treg), including the naturally occurring CD25<sup>+</sup>FoxP3<sup>+</sup> Treg in the thymus and the adaptive Treg in periphery, are responsible for maintaining tolerance to self-antigens and controlling excessive immune response to external antigens [7]. The potential mechanisms of MSC-induced Treg differentiation may involve (i) direct cell–cell contacts, (ii) the production of prostaglandin E2 and transforming growth factor  $\beta$ -1 (TGF $\beta$ -1) and (iii) the release of a non-classical HLA class I molecule, HLA-G5 [8]. In addition, micro-vesicles derived from MSC may help transfer cellular materials, including RNA and proteins, and organelles to neighbouring cells [9–11]. Interestingly, various *in vitro* observations suggest that the culture conditions, the types and concentrations of cytokines in the milieu and the activation status of T cells at the time of exposure to MSCs also influence their final differentiation [12]. In addition to their impact on T-cell fate, MSCs influence macrophage outcomes, with a preferential shift towards an anti-inflammatory immunosuppressive M2 phenotype [13, 14]. M2 macrophages have been implicated in the generation and maintenance of Treg. Finally, MSC treatment *in vitro* inhibits antigen-presenting cells (APC), which further favours Treg expansion through the release of TGF $\beta$  [15]. *In vivo*, the beneficial MSC-induced polarization of T cells towards a Treg phenotype has been demonstrated in numerous experimental models of autoimmune and inflammatory diseases, such as systemic lupus erythematosus, fibrillin-mutated systemic sclerosis or colitis.

In addition to these immunoregulatory properties, MSC exert tissue repair functions in damaged organs [16]. In particular, experimental observations have demonstrated their protective effect in AKI, as detailed *infra*. Following a renal IRI, MSCs reduce inflammation and accelerate vascular supply [17]. Indeed, single or repeated injections of MSC or MSC-derived micro-vesicles after injury accelerate functional recovery of the kidneys [16], and improve survival in a lethal model of AKI [10]. MSC activate endogenous cellular repair programmes by releasing various growth factors such as fibroblast

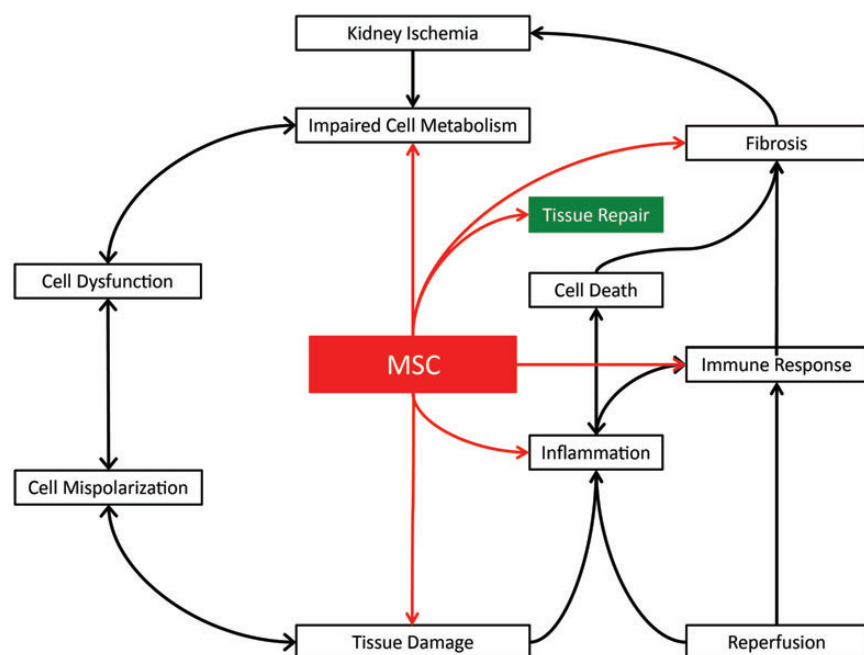
growth factor, hepatocyte growth factor, insulin-like growth factor (IGF), keratinocyte growth factor, monocyte chemoattractant protein-1, stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor. Additional MSC-related mediators, including IL-10, IL-6, TGF- $\beta$  or nitric oxide (NO), may further facilitate a local anti-inflammatory state, thereby allowing the healing of damaged tissues. Moreover, the expansion of surviving renal tubular cells observed with the administration MSC or MSC derivatives result from the induction of pro-survival genes and down-regulation of pro-apoptotic genes. Finally, MSC-derived micro-vesicles help to rapidly restore adenosine triphosphate (ATP) supply following IRI by transferring mitochondria into the damaged cells [10, 11]. Such tissue repair effect of MSC therapy in AKI has also been successfully observed in other organs, like the liver, lungs, heart and intestines [16].

Altogether, these *in vitro* and *in vivo* observations highlight the significant potential of immunomodulation, anti-inflammation and tissue repair of MSC via both direct cell–cell interactions and the release of paracrine factors.

## PATHOPHYSIOLOGY OF RENAL I/R

A sustained interruption of kidney perfusion is associated with a rapid drop in oxygen partial pressure and nutrient concentration, which leads to tissular and cellular events eventually causing 'ischaemic damage' [2, 3]. At the cellular level, ischaemia causes a rapid depletion of the energy supply, i.e. ATP, of renal tubular cells since oxidative phosphorylation can no longer proceed in the mitochondria in the absence of oxygen (Figure 1). Anaerobic glycolysis transiently allows a residual production of ATP, but is associated with the intracellular accumulation of lactate and the acidification of cell cytosol, which further perturbs mitochondrion and cell functions. In addition, the lack of energy delivery induces the disorganization of the cytoskeleton, the disruption of intercellular tight junctions, the loss of cell polarity and the dysfunction of membrane ion transporters, including the Na<sup>+</sup>/K<sup>+</sup>-ATPase [2, 18]. Consequently, epithelial and endothelial cells detach from their basal membrane and obstruct tubular and vascular lumens, respectively. The loss of tubular and vascular permeability causes a major accumulation of fluids in the interstitium, which further delays kidney reperfusion and prolongs the ischaemic insult. Moreover, renal cell injury caused by I/R is associated with a massive and local production of reactive oxygen species (ROS), which are responsible for the detrimental oxidation of proteins, lipids, membranes and nucleic acids of both epithelial and endothelial cells [19].

In addition to all these metabolic consequences, IRI is associated with a significant inflammatory reaction characterized by the expression and activation of endothelial adhesion molecules, integrins and selectins. Such 'sterile inflammation' is characterized by the release of danger-associated molecular pattern molecules, which, in turn, activate the innate immune responses via the Toll-like receptors, and recruit inflammatory cells [20]. The deleterious impact of I/R-associated inflammation and infiltration of monocytes involves chemokine



**FIGURE 1:** Schematic view of the putative benefits of mesenchymal stromal cells (MSC) on renal ischaemia/reperfusion injury (IRI). MSC may modulate the inflammatory and immune response and restore cell metabolism, thereby attenuating tissue damage and accelerating the regeneration process. The mechanisms of MSC therapy in renal IRI are detailed in text.

receptors, such as chemokine receptor-2, chemokine receptor-7, CXC chemokine receptor-4 as well as the local production of ROS, tumour necrosis factor- $\alpha$  and interleukin-1 $\beta$  [21]. In addition, there is a sustained amplification of IgG<sub>1</sub> antibodies directed against an antigen encountered in the days following renal I/R [22]. Interestingly, the total amount of antigen-unspecific IgG<sub>1</sub> and the number of B lymphocytes remain unchanged during this period, but the number of antigen-specific lymphocytes increases. This effect is lost in mice deficient in complement factor B that lacks a functional alternative pathway of complement, as well as in IL10-deficient mice. These observations suggest that kidney IRI leads to a rise in antibody production against heterologous antigens [22]. Still, the role of B lymphocytes at the time of IRI remains unclear, with conflicting observations as to whether these cells are protective or harmful to the ischaemic kidney [23, 24]. Furthermore, the activation of naïve T cells through antigen presentation by dendritic cells may contribute to the enhanced kidney immunogenicity following IRI [25]. All these inflammatory and immune consequences may play an even more important role in renal I/R at the time of KT, as detailed *infra*. A better understanding of the tissular and cellular phenomena associated with renal IRI would thus help exploit them to prevent or attenuate the ischaemic damage.

### MSC THERAPY IN RENAL IRI

MSC are characterized by significant properties of immunomodulation, anti-inflammation and tissue repair, which could be advantageous against renal IRI [16]. Indeed, MSC therapy may act all along the IRI process, from attenuating ischaemia-related metabolic disturbances to preventing reperfusion-associated

inflammation and facilitating tissue regeneration (Figure 1). As summarized above, both direct cell–cell interactions and the release of paracrine or endocrine factors have been described in MSC therapy. Furthermore, the sole administration of MSC derivatives, like micro-vesicles and conditioned media, has also demonstrated beneficial effects in distinct models of renal IRI.

To the best of our knowledge, the administration of MSC or MSC derivatives in most, if not all, experimental protocols of MSC therapy in renal IRI occurs after the injury [16]. The impact of MSC in renal ischaemic conditioning (IC) remains thus ill-defined. The principle of IC consists in exposing an organ to repeated episodes of brief (3–5 min) ischaemia by arterial clamping/unclamping before a prolonged ischaemia. Numerous experimental models using the heart, intestine, brain, liver or kidneys have shown that IC leads to a low-energy tissular and cellular status, which helps preserve cell metabolism and architecture, reduces inflammation at the time of reperfusion and accelerates functional recovery of the organ [26, 27]. Interestingly, renal IC can also be achieved by applying brief and repeated episodes of I/R to a remote organ, like the limbs or the intestine [28]. The mechanisms of remote IC remain poorly characterized and may involve the autonomous neurogenic cascade, as well as the secretion of diverse biochemical messengers into the bloodstream [20, 28, 29]. Characterizing the signal transduction pathways involved in both direct and remote IC is urgently required to develop and validate non-invasive approaches for IC. Indeed, fractionated episodes of ischaemia surgically performed on the renal arteries might damage the vascular structures and/or induce platelet aggregation with micro-embolization into the renal parenchyma, thereby worsening reperfusion. Various targets have been pharmacologically tested in rodent models of renal

IRI, as summarized in Table 1. Whether MSC therapy represents a safe non-invasive method for renal IC remains to be determined.

In anaesthetized rats with I/R-induced AKI, the intra-carotid administration of MSC either immediately or 24 h after renal IRI resulted in significantly improved renal function, higher proliferative and lower apoptotic indexes, as well as lower renal injury and unchanged leucocyte infiltration scores [30]. Using *in vivo* two-photon laser confocal microscopy, fluorescence-labelled MSC could be detected in glomeruli within the first hour after cell infusion. Cell migration and recruitment to the injured tissue may follow an SDF-1/CXCR4 (stromal cell-derived factor 1) and CXCR7 gradient [31]. However, within 3 days of administration, none of the administered MSC had differentiated into a tubular or endothelial cell phenotype. Similarly, a C57BL/6 mouse model of liver IRI infused with C57BL/6 MSC constitutively expressing DsRed-fluorescent protein and radioactively labelled with Cr-51 demonstrated that MSC are short-lived and that viable MSC do not pass the lungs. Thus, long-term beneficial effects of MSC appear primarily mediated by paracrine actions and not by their engraftment and trans-differentiation into target cells [32]. Several experimental models using different animal species have suggested that the beneficial effect of MSC on renal tubular cells proliferation following IRI may be linked to their ability to suppress oxidative stress and attenuate the inflammatory response [33, 34]. At later times after injury (i.e. 6–10 weeks), MSC therapy also improves functional parameters and reduces progression of renal fibrosis, with reduced mRNA expression of type I collagen and vimentin [35].

Such a global protective effect appears to be mostly mediated by modulation of the inflammatory response and/or hypoxia, which blocks the epithelial-mesenchymal transition. Various experimental manipulations have successfully been conducted to further enhance the protective ability of MSC in renal IRI, including genetic overexpression of BMP-7 or HGF and co-administration with pharmacological agents. Hence, the concomitant application of MSC and darbepoetin- $\alpha$  after

IRI in rats leads to reduced tissue injury and better renal recovery in comparison with MSC or darbepoetin- $\alpha$  alone [36]. In addition, *in vitro* hypoxic conditioning of MSC before their infusion ameliorates their recruitment and viability into the injured tissue, as well as their tissue repair capabilities via an increased secretion of pro-angiogenic, anti-apoptotic and mitogenic factors. Neutralization of either CXCR4 or CXCR7 impaired the improved therapeutic potential of MSC cultured in hypoxic conditions [31]. Similarly, MSC preconditioning with type 1 IGF (IGF-1) before administration improves cell migration capacity and restores normal renal function after AKI [37].

Besides direct cell-cell interactions, the renal protective efficacy of MSC may involve paracrine factors inducing resistance to apoptosis and favouring proliferation of surviving tubular cells [38]. A single administration of MSC-derived micro-vesicles immediately after IRI protects rats from AKI by inhibiting apoptosis and stimulating tubular epithelial cell proliferation, thereby significantly reducing the impairment of renal function and preventing chronic renal damage. In an alternative model of glycerol-induced AKI in SCID mice, the sole infusion of micro-vesicles derived from human bone marrow MSC accelerated morphologic and functional kidney recovery to a similar extent as classical MSC therapy. MSC-derived exosomes are thought to vehicle a specific subset of cellular mRNA implicated in the mesenchymal phenotype and in the control of transcription, proliferation and immunoregulation [39].

On the basis of these promising observations in animal models of renal IRI, clinical studies using allogenic or autologous MSC in the settings of ischaemic AKI at the time of cardiac surgery have been initiated ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). According to preliminary results of phase I and II trials involving MSC therapy (#NCT00733876; #NCT01602328), no major adverse events have been reported. Still, essential questions remain to be addressed, including the route of delivery, the adequate number/volume of MSC infusion, the timing and frequency of MSC administration before and/or after renal IRI and the clinical and immunological tolerance to these cells [16]. No data regarding the impact of MSC therapy on kidney protection and the eventual regeneration following AKI are currently available in man.

**Table 1. Drug-mediated renal ischaemic conditioning in rodent models**

Pathways	Drugs	Intracellular targets
Hypoxia	Erythropoietin Isoflurane	HIF-1 $\alpha$
Oxidative Stress	N-acetyl-cysteine L-carnitine Danshen Ethanol Lithium	Reactive oxygen species NOS
Inflammation	Spermine NONOate Ciclosporine; tacrolimus Adenosine (and agonists) Apyrase (CD39 soluble) Catecholamine's	MAPK GPCR
Metabolism	AICAR; Metformin; Resveratrol Hemin	AMPK Hmox1 inducer

HIF1, hypoxia-inducible factor 1; AMPK, AMP-activated protein kinase; NOS, nitric oxide synthase; MAPK, mitogen-activated protein kinases; GPCR, G-protein-coupled receptors.

## MSC THERAPY IN THE SPECIFIC SETTING OF KT

KT represents the best treatment for patients with end-stage renal disease. According to the latest reports from EuroTransplant ([www.eurotransplant.org](http://www.eurotransplant.org)), graft survival rates at 1, 3 and 5 years following KT from a deceased donor are 84, 78 and 68%, respectively. When living donors are considered, graft survival reaches 93, 89 and 82% at 1, 3 and 5 years following KT. The main causes of graft loss are acute rejection (AR) and chronic allograft nephropathy (CAN). Although encouraging, these numbers about graft survival reflect the possibility to further improve KT efficiency, and emphasize the interplay between immune and non-immune factors. Indeed, KT necessarily delivers a major histocompatibility complex-



incompatible graft from a donor to a recipient. This simultaneously triggers the activation of both graft-destructive effector T cells and protective Treg. The outcome of the renal graft mostly depends on the balance between such opposing cell populations. Consequently, current research programmes further investigate the immune cascades at the time of KT, with particular focus on the role of complement factors, IL-10 and B and Treg cells. In addition, the optimal timing for interventions modulating the humoral response still needs to be addressed. The unfortunate consequences of renal I/R are cell death and tubular atrophy as well as irreversible deposits of collagen in the interstitium. This condition, formerly described as CAN, is associated with a sustained status of hypoxia and a shortened duration of graft survival [40]. There is evidence that CAN is also related to low-grade antibody-mediated rejection as well as to antibody-independent functions of B cells (i.e. antigen presentation, lymphoid neogenesis). In addition to these acute and chronic immune events, long-term graft survival is also influenced by the quality of the graft at the time of its transplantation, and particularly by the level of ischaemic damage caused by transplantation *per se*. Indeed, IRI represents an unavoidable event during solid organ transplantation (SOT), and significantly participates to early graft dysfunction and enhanced graft immunogenicity [16, 41].

On the basis of the immunomodulatory, anti-inflammatory and tissue repair properties of MSC, such a therapy has been proposed in the field of SOT. Indeed, MSC could in theory attenuate the consequences of both IRI and AR following transplantation, as well as prevent long-term allograft pathology. A large number of animal transplant studies using MSC or MSC derivatives have demonstrated that such therapy improves the outcome of SOT via both paracrine and cell-dependent mechanisms of action [41, 42]. In the particular field of KT, MSC were shown to ameliorate inflammation caused by prolonged cold ischaemia [43]. However, the best timing of MSC infusion capable of promoting long-term immune tolerance without impairing early graft function remains controversial [44, 45]. In a highly mismatched donor–recipient rat KT model, preoperative application of MSC resulted in enhanced humoral immune responses, increased inflammation and rejection and higher degree of kidney cortex tissue damage [44]. In contrast, in a mouse model of fully allogeneic Balb/c kidney transplant in C57 recipients presensitized by donor cell infusion, MSC infusion prior to KT induced a significant prolongation of kidney graft survival by early expansion of Tregs in lymphoid organs [45]. In this particular mouse model characterized by a high frequency of donor-reactive memory T cells, MSC therapy after KT caused premature graft dysfunction, with an increased recruitment of neutrophils and complement C3 deposition into the graft. These observations raise questions regarding the best timing for MSC administration at the time of KT, with the dual hazard of MSC pre-sensitization and/or MSC-associated engraftment syndrome. In a rat model of CAN, MSC therapy prevented long-term interstitial fibrosis and tubular atrophy [46].

Following on these promising observations from pre-clinical models, clinical trials were designed to assess the putative benefits of MSC therapy in human transplant recipients, with

a particular focus on graft outcomes and patient quality of life. The Mesenchymal Stem Cells in Solid Organ Transplantation (MiSOT) Consortium was founded to enable effective collaboration between research groups working in the application of adherent stem cell products in SOT. The 2012 MiSOT report includes six clinical trials applying MSC in SOT. MSC administration in clinical transplantation has proved relatively safe and feasible. Preliminary data indicate that MSC may (i) prevent AR, (ii) induce systemic alloimmune modulation and (iii) reduce induction and maintenance immunosuppressive regimens [47]. MSC administration also attenuates tubulitis and interstitial fibrosis/tubular atrophy in some patients, thereby favouring long-term stable graft function. However, several issues, including proper timing, concomitant immunosuppression, source and immunogenicity of MSC and their potential oncogenicity, remain to be addressed before advising a broader application of MSC therapy in SOT [47]. In particular, lessons can be learned from the tailor-made step-wise approach of Remuzzi *et al.* [48, 49]. In a pilot study of safety and feasibility, two living-related donor kidney recipients were given autologous MSC ‘at day 7 post-transplant’, after induction therapy with basiliximab/low-dose thymoglobulin [48]. In both patients, MSC therapy caused a pro-tolerogenic environment characterized by lower CD8<sup>+</sup> T cells, higher CD4<sup>+</sup> Treg and reduced donor-specific CD8<sup>+</sup> T-cell cytotoxicity. However, both MSC-treated patients developed AKI early following MSC infusion, with graft recruitment of neutrophils and *in situ* complement-C3 deposition [48]. The authors hypothesized that KT surgery triggered graft inflammation causing an engraftment syndrome characterized by a local recruitment and activation of MSC towards a pro-inflammatory phenotype. In a second clinical attempt, two living-related kidney recipients were infused with autologous MSC 24 h before KT [49]. In addition, the anti-IL-2-receptor monoclonal antibody, basiliximab, was removed from the induction regimen since it had been associated with a transient decrease of circulating CD25<sup>+</sup>FoxP3<sup>+</sup> regulatory Treg. In this clinical setting, no engraftment syndrome was observed in the early post-transplant phase. However, one patient developed AR 2 weeks post KT [49]. The expansion of CD25<sup>+</sup>FoxP3<sup>+</sup> Treg was similar in MSC-treated patients with or without basiliximab induction. Altogether, these pilot MiSOT clinical trials will help further amend MSC protocol in KT, thereby defining the most favourable conditions for long-term tolerance while circumventing early adverse effects.

## PERSPECTIVES

AKI represents a worldwide public health issue of increasing incidence, which is associated with a significant morbidity and mortality. Treatment of patients presenting with AKI mostly relies on supportive manoeuvres, in the absence of specific target-orientated therapy. The pathophysiology of AKI commonly involves IRI, which leads to both immune and metabolic consequences in renal tissue. Because MSC have demonstrated immunomodulatory and tissue repair properties, their administration at the time of AKI and/or at later

times could attenuate IRI severity and accelerate the regeneration process. Their impact on kidney IC, i.e. before IRI, remains to be assessed. Even more promising, MSC derivatives have proved efficient in animal models of AKI, which further emphasizes the role of paracrine mediators in MSC therapy and may help avoid total cell infusion. In SOT, MSC could help cure both IRI and AR, thereby limiting graft immunogenicity and increasing its function and survival. MSC therapy in animal models and in pilot clinical trials shows encouraging results and opens novel avenues in the management of both AKI and KT.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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