Progress toward the clinical application of mesenchymal stromal cells and other
disease-modulating regenerative therapies: Examples from the field of nephrology

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Abstract

Drawing from basic knowledge of stem cell biology, embryonic development, wound healing and aging, regenerative medicine seeks to develop therapeutic strategies that complement or replace conventional treatments by actively repairing diseased tissue or generating new organs and tissues. Among the various clinical translational strategies within the field of regenerative medicine, several can be broadly described as promoting disease resolution indirectly through local or systemic interactions with a patient’s cells without permanently integrating or directly forming new primary tissue. In this review, we focus on such therapies, which we term disease modulating regenerative therapies (DMRT), and on the extent to which they have been translated into the clinical arena in four distinct areas of nephrology: renovascular disease (RVD), sepsis-associate acute kidney injury (SA-AKI), diabetic kidney disease (DKD) and kidney transplantation (KTx). As we describe, the DMRT that has most consistently progressed to human clinical trials for these indications is mesenchymal stem/stromal cells (MSCs), which potently modulate ischemic, inflammatory, pro-fibrotic and immune-mediated tissue injury through diverse paracrine mechanisms. In KTx, several early-phase clinical trials have also tested the potential for ex vivo-expanded regulatory immune cell therapies to promote donor-specific tolerance and prevent or resolve allograft injury. Other promising DMRT, including adult stem/progenitor cells, stem cell-derived extracellular vesicles and implantable hydrogels/biomaterials remain at varying pre-clinical stages of translation for these renal conditions. To date (2021), no DMRT has gained market approval for use in patients with RVD, SA-AKI, DKD or KTx and clinical trials demonstrating definitive, cost-effective patient benefits are needed. Nonetheless, exciting progress in understanding the disease-specific mechanisms of action of MSCs and other DMRT, coupled with increasing knowledge of the pathophysiological basis for renal tissue injury and the experience gained from pioneering early-phase clinical trials provide optimism that impactful, regenerative treatments for diverse kidney diseases will emerge in the years ahead.
Introduction

Since it was first introduced into biomedical parlance by William Haseltine, the term regenerative medicine has become broadly recognizable to healthcare providers and the general public alike. Following two decades of intense interest and diverse research initiatives, the original concept of “an approach to therapy that...employs human genes, proteins and cells to re-grow, restore or provide mechanical replacements for tissues that have been injured by trauma, damaged by disease or worn by time" still conveys a succinct and valid definition of the field.(1) Critically, one of the central tenets of regenerative medicine has been the merging of basic insights into organ/tissue development, stem cell science and disease pathophysiology with innovative translational concepts and manufacturing procedures to reverse disease more effectively than can currently be achieved by conventional pharmacotherapy and interventional procedures.(1, 2)

For the nephrologist, the promise of regenerative medicine is compelling. Most acute and chronic kidney diseases remain incurable, life-limiting and are typically managed with drug combinations or procedures that are costly and carry a high burden of adverse effects. Added to this is the nephrologist’s natural affinity for the application of cellular and physiological science to patient management. In this article, we describe recent progress within a specific aspect of regenerative medicine, which we term “disease-modulating regenerative therapies” (DMRT), in the field of nephrology. In focusing on DMRT, we specifically refer to therapeutic concepts based on systemic or localized administration of cells, subcellular components, biomaterials or combinations of these which engage in a complex molecular crosstalk with resident cells and tissues of the host to modify or reprogram damaging biological activity. We distinguish DMRT from other regenerative strategies that are based on harnessing pluripotent/multipotent stem cells and advances in tissue engineering to directly repair or replace damaged organs and tissue. This aspect of regenerative medicine, which might be termed “organ and tissue-replacing regenerative
First and foremost among DMRT are mesenchymal stem/stromal cells (MSCs), the subject of two decades of translational research, which have been administered safely to patients in numerous clinical trials.(6, 7) Critically, MSCs are now considered to mediate their therapeutic benefits predominantly through inducible secretion of paracrine mediators and reprogramming of myeloid and lymphoid immune cells and can be culture-expanded to large numbers from a range of autologous or allogeneic tissue sources.(6) In addition to MSC-based cell therapies, other forms of DMRT have made varying degrees of progress along a similar translational path: (a) Regulatory T-cells (T-reg) and other immunological cells which suppress inflammation or harmful immune responses,(8, 9) (b) Additional types of adult progenitor cells (including those derived from the kidney) with pro-repair paracrine effects,(10, 11) (c) Stem/progenitor cell-derived extracellular vesicles (EV) which may transfer pro-regenerative biomolecules to target cells,(12) and (d) Injectable hydrogels and other biomaterials which may have inherent regenerative properties or serve to enhance the benefits of cell-based therapies.(13) As summarized in Figure 1 and described in detail in subsequent sections of the review, we focus on the progress toward clinical translation of MSCs and other DMRT that has occurred to date in four important areas of clinical practice in nephrology – renovascular disease (RVD), sepsis-associated acute kidney injury (SA-AKI), diabetic kidney disease (DKD) and kidney transplantation (KTx). For each of these exemplars, we highlight the extent to which early-phase clinical trial experiences with DMRT are being driven by increased understanding of their potential mechanisms of action and are linked to advances in knowledge of the pathophysiological basis of the targeted condition.

Renovascular disease

Hypertension is a major risk factor for chronic kidney disease (CKD) with hypertensive kidney disease accounting for approximately 30% of all end stage of kidney disease (ESKD) cases.(14) Hypertensive kidney disease, characterized by vascular damage, endothelial
dysfunction, and loss of endogenous vasodilators, results in progressive loss of the renal microvasculature.(15) Renovascular disease is a common cause for secondary hypertension in individuals aged 65 years and older and RVD attributed to atherosclerotic plaque development with reduction in renal artery dimension, represents a unique intersection between hypertension and CKD/ESRD leading to progressive renal insufficiency.(16) In the setting of significant RVD, further reduction of renal blood flow and hypoxia trigger inflammation, oxidative stress and pro-fibrotic pathways, leading to scarring and further deterioration of renal function (ischemic nephropathy).(17) Importantly, recent clinical trials and experimental studies indicate that restoration of large vessel patency alone is not enough to regain kidney function in most patients with atherosclerotic RVD.(18, 19) The limited number of currently-available strategies for effectively modulating RVD and the realization that the natural history of this disease involves transition from a hemodynamic component to a pro-inflammatory and pro-fibrotic disease highlight the need for a paradigm shift in therapy.(20) Importantly, RVD-associated ischemic nephropathy and hypertensive kidney disease, in the absence of RVD, share common pathophysiological features which include activated renin-angiotensin system, increased sodium retention and, consequently, increased oxygen consumption.(21) In evidence of this, studies in hypertensive rats using oxygen microelectrodes found pronounced medullary and cortical hypoxia in spontaneously hypertensive compared to normotensive controls.(22) Furthermore, the presence of ischemic, rather than hypertrophic, glomeruli in hypertensive kidney disease suggests that hypoxia and ischemia are the predominant mechanisms.(23) Therefore, in subjects with prolonged hypertension and relevant genetic, environmental and lifestyle-related risk factors, limited blood flow and oxygenation to areas of renal parenchyma where the oxygen tension is below 10 mm Hg makes the kidney vulnerable to ischemic injury which resembles that due to RVD. In this context, implementation of DMRT, in particular the use of MSCs, can be viewed as a novel therapeutic option for RVD as well as hypertensive kidney disease.
Importantly, MSCs have immunomodulatory, anti-inflammatory and proangiogenic properties which have been demonstrated in experimental studies in animals and humans with RVD.(24-26) Arterial delivery of MSCs in the swine model of RVD was associated with protection of the stenotic kidney and improved renal blood flow and function with reduction of oxidative stress and inflammation, contributing to tissue repair.(24) The strong paracrine effect of MSCs, and not their differentiation capacity, seems to be the principal mechanism of their therapeutic action. In clinically-relevant, large animal models of RVD, MSCs have been shown to release a variety of soluble mediators that act locally within the kidney to ameliorate ischemic nephropathy through pro-angiogenic, anti-inflammatory and anti-oxidative mechanisms.(24) Also consistent with a paracrine model, the therapeutic potential of stem/progenitor cell-derived EVs in renal diseases has been highlighted by showing decreased renal inflammation and injury through intrarenal delivery of EVs in pigs with RVD and concomitant metabolic syndrome.(27) Other studies have demonstrated their role as carriers of anti-inflammatory genes and proteins and their capacity to be engineered to deliver specific substances or to have enhanced uptake by target cells.(28-30) Therefore, MSC-derived EVs may serve as an acellular therapeutic option to attenuate inflammation and fibrosis in RVD and other forms of renal disease. As illustrated in Figure 2 for the clinical target of RVD and associated ischemic nephropathy, the paracrine regenerative activities of MSCs, including both secreted soluble mediators and released EVs, are now recognized to be “tunable”. Thus, as described in greater detail later, disease-associated dysfunction of ex vivo-expanded autologous MSCs may be reversed through hypoxic preconditioning and other manipulations.(31, 32)

There is now promising evidence that observations of RVD modulation by MSCs in animal models can be translated into clinical benefits. In a Phase I trial in patients with RVD, we (LJH, SMH) recently demonstrated that intra-arterial infusion of autologous adipose tissue-derived MSCs into post-stenotic kidneys (without concomitant renal artery angioplasty) resulted in increased cortical renal blood flow (RBF) and glomerular filtration rate (GFR)
compared to the baseline values. These improvements in hemodynamic and functional indices were associated with attenuation of tissue hypoxia, inflammatory cytokines and angiogenic biomarkers as well as a fall in blood pressure between baseline and 3 months follow-up. These changes were more prominent in the patients treated with higher MSC dose.(26, 33) Of further interest, we also observed RBF increases in the (non-MSC-infused) contralateral kidneys.(26, 33) This phenomenon likely occurred due to “cross-talk” signalling between kidneys and/or wider systemic effects of signalling and homing signals for MSCs. Moreover, beneficial off-target effects beyond the kidney are also possible as infusion of MSCs or endothelial progenitor cells into the renal artery has been associated with attenuation of hypertensive cardiomyopathy in experimental models of renovascular hypertension.(34) Despite the promising evidence base of pre-clinical and clinical application of MSCs and MSC-derived EVs in RVD, there has been limited exploration to date of alternative DMRT such as regulatory immune cells, other adult progenitor cells and implantable hydrogels and in this area (Figure 1). Nonetheless, experimental evidence in models of renal ischemia reperfusion injury could provide a basis for future translation of such therapies for chronic ischemic nephropathy in the future.(13, 35, 36)

Sepsis-Associated Acute Kidney Injury

Acute kidney injury has a prevalence ranging from 1-20% of hospitalized patients and 50-60% of patients in the intensive care unit. Mortality is proportional to the severity of AKI and 30% of survivors die within the first year after hospital discharge. Sepsis, which is the most frequent cause of AKI in the critically ill,(37) is a life-threatening syndrome resulting from a disordered immune response to uncontrolled microbial infection.(38) The pathophysiology of sepsis is dominated by dysregulated inflammation and immune suppression, with endothelial and epithelial injury, leukocyte aggregation, mitochondrial dysfunction, apoptosis and impaired regeneration.(37) Sepsis-associated AKI differs from ischemic and toxic AKI, being characterized by global renal hyperaemia with altered renal blood flow distribution and inflammation incited by both infiltrating immune cells and resident parenchymal cells.(37) In
SA-AKI, there is an abnormal repair process due to prolonged hypoxia, cytokine expression, and defective adaptive immune cell function. Critically ill patients with persistent or recurrent AKI are at very high risk for secondary infection and increased mortality and represent a key target for novel and more effective therapies.(39) In this regard, MSCs have demonstrated benefits in multiple sepsis models, including lipopolysaccharide administration, bacterial pneumonia and polymicrobial abdominal sepsis.(40-42) In animal models of sepsis, MSC administration is reportedly associated with improved survival, reduced organ injury, increased clearance of bacteria, cells and fluid, and resolution of inflammation.(43-45) Some animal model studies of SA-AKI have demonstrated improvement in tubular injury scores and kidney function(42, 45) while others have not(43, 44) – an inconsistency that may reflect differences among the models used.

From a mechanistic perspective, it is now clear that MSCs administered intravenously in models of sepsis localize predominantly in the lungs. From this location, they mediate their systemic benefits through mechanisms involving cross-talk with immune cells that result in modulatory effects on cytokine expression, vascular permeability, removal of apoptotic cells and clearance of bacteria by neutrophils and macrophages (Figure 3).(46) In addition to release of soluble mediators and reprogramming of immune cells by viable MSCs, it has recently been shown that disease modulation may occur as a result of the induction of apoptosis of intravenously-infused MSCs by cytotoxic lymphocytes followed by their phagocytosis by resident myeloid cells (monocytes and macrophages). This process, referred to as efferocytosis, results in polarization of myeloid cells toward alternatively-activated (M2-like) phenotypes with potent anti-inflammatory effects.(47, 48) Furthermore, either directly or through their effects on myeloid cells, MSCs also augment tissue repair processes through promoting expansion of regulatory T cells (T-reg).(49)

A number of specific soluble factors have been identified as mediating the paracrine effects of MSCs and their immune cell partners in models of sepsis. These include interleukin (IL)-10, keratinocyte growth factor, prostaglandin E2 (PGE2), vascular endothelial growth factor
(VEGF), antibacterial peptides LL-37 and hepcidin and other pro-resolution factors. The role of IL-10 has been most convincingly demonstrated. Nemeth et al. first reported in the mouse cecal ligation and puncture model of polymicrobial sepsis that intravenously administered MSCs stimulate IL-10 production by macrophages through PGE2/EP2 receptor interaction resulting in the prevention of neutrophil extravasation into tissue. This MSC-induced pulse of IL-10 production has since been replicated in several other studies. Transfer of specific micro (mi)RNAs or mitochondria via nanotubes may also underlie some of the effects of MSCs to enhance macrophage phagocytic activity or endogenous stem cell fitness in the septic environment. A further strategy to enhance the immunomodulatory features of MSCs in sepsis is through pre-conditioning (“licensing”) with pro-inflammatory cytokines, toll-like receptor ligands, carbon monoxide and eicosapentaenoic acid. In the case of carbon monoxide licensing of MSCs, this was reported by Tsoyi et al. to result in reduced organ damage (including kidney injury) and superior survival in mouse models of sepsis while also allowing for later MSC administration. Mechanisms by which pre-conditioning has been reported to enhance MSC activity include activation of the lipoxygenase pathway and enhanced exosome delivery of miRNA to macrophages. As already described in the context of RVD, MSC-derived EVs also have the potential to be developed as a subcellular DMRT for sepsis and SA-AKI through the transfer of a wide range of bioactive molecules.

Although the clinical application of MSCs in sepsis and SA-AKI is at an early stage, they have shown promising safety profiles in early-phase human trials. For example, in a Phase 1 dose-escalation trial involving 9 patients with sepsis, treatment with MSCs was found to be safe and well tolerated albeit with no overt effect on sepsis parameters. An analysis of cytokine levels in treated patients demonstrated no increase in known pro-inflammatory mediators or biomarkers of organ dysfunction following MSC treatment. Swaminathan et al., in a phase 2 multi-center randomised, placebo-controlled clinical trial examined the effect of allogeneic MSC therapy delivered intra-aortically in patients
undergoing cardiac surgery who developed post-bypass AKI. More than half of these patients had impaired renal function at baseline. (58) While this trial was carried out in patients with a sterile form of AKI, the design and results have important implications for the future planning of clinical trials of DMRT in SA-AKI. Disappointingly, in this trial, MSC administration resulted in no difference in recovery of renal function, dialysis or death compared to placebo. (58) Although carried out in a relatively homogenous patient population, differences in renal reserve, complexity of cardiac surgical procedure, bypass time and other post-operative complications may yet have hindered the ability to observe any modest clinical benefit of MSCs. This negative study in sterile AKI does not necessarily blunt interest in the clinical translation of MSCs for SA-AKI. Indeed, trials of MSCs and other DMRT in sepsis may be better suited to detecting their effects on the development or severity of AKI. As sepsis is a multi-organ disorder, favourable effects of systemically administered MSCs on kidney function may derive from improved function of other organ systems and from reprogramming of immune cells at distant sites. Indeed, preclinical studies indicate that infusion of apoptotic versus viable MSCs within the lung led to greater suppression of inflammation, oxidative stress, cellular markers of immune reactivity and a less marked kidney injury in a caecal ligation and perforation model of sepsis. (59) Given the high prevalence of AKI among patients with sepsis and its implications for morbidity and mortality, it will be important for future clinical trials of DMRT in sepsis to include patients with or at risk for developing AKI, for equal number of patients with similar stages of AKI to be randomized and for specific renal end-points to be included in the trial design.

**Diabetic Kidney Disease**

Due to the growth of the aging population, the number of individuals with diabetes mellitus (DM) worldwide increased from 108 million in 1980 to 422 million in 2014. (60) Moreover, the global epidemic of DM has contributed approximately 50% of the increased health burden due to CKD. (61) Diabetic kidney disease is characterized by vascular damage resulting from cumulative effects of a wide range of predominantly hyperglycemia-driven maladaptive
processes including chronic inflammation, increased oxidative stress, advanced glycation end-product accumulation, steatosis, insulin resistance, renal hypoxia, apoptosis, cellular de-differentiation and senescence, and altered renin-angiotensin-aldosterone system (RAAS) activation. (62-64) Intrinsic renal regenerative capacity is limited in DM, exacerbating chronic glomerulosclerosis, tubulointerstitial fibrosis, and chronic inflammation. (62, 64, 65) Although recent clinical trials of SGLT2 inhibitors and other pharmacological agents have shown that the rate of renal functional loss can be slowed in DKD due to type 2 DM, (66-68) successful targeting of multiple injurious pathways such as those mediating inflammation, oxidative stress, renal hypoxia and fibrosis may be necessary to truly halt DKD.

With this goal in mind, DMRT represent novel therapeutic options for the delivery or induction of a wide range of mediators to simultaneously target maladaptive processes that contribute to DKD progression. As with other renal diseases, the most extensively studied DMRT in DKD is the MSC. (64) In many pre-clinical experimental models of DM and diabetic nephropathy, (64, 68) the paracrine-mediated actions and cell-cell interactions of exogenously-administered MSCs have shown potential to modulate a range of pathophysiological processes that contribute both locally and systemically to the progressive renal injury and functional loss that characterize DKD (Figure 4). External to the kidneys, MSCs delivered intravenously or by other routes have been experimentally shown in models of DM to modulate adipose tissue inflammation, preserve islet function and enhance insulin sensitivity – leading indirectly to beneficial renal effects through reducing glycemia and the pro-inflammatory systemic environment. (64, 69, 70) Concomitantly, MSCs themselves, their released mediators and regulatory immune cells induced as a result of MSC administration may transfer to the kidneys to mediate beneficial effects within distinct renal compartments including the glomerulus, microvasculature, tubules and interstitium. Reductions in glomerular size, podocyte apoptosis, glomerular matrix expansion/sclerosis, peritubular interstitial fibrosis, renal tubular epithelial cell death and de-differentiation, tubulo-interstitial
fibrosis, and microvascular rarefaction have been observed in association with reduced albuminuria and stabilization of glomerular filtration rate. (64, 69, 70)

Pre-clinical MSC-based experimental studies have demonstrated benefits derived from a variety of therapeutically-relevant mediators (Figure 4). These include soluble factors with anti-fibrotic [hepatocyte growth factor (HGF)], pro-angiogenic (VEGF), anti-apoptotic/homeostasis [HGF, VEGF, stromal cell derived factor (SDF-1/CXCL-12)] and immunomodulatory [indoleamine 2,3 dioxygenase (IDO), prostaglandin-E2 (PGE2) and interleukin (IL)-10] activity. Such soluble factors may be secreted inherently by MSCs, triggered in MSCs by signalling from pro-inflammatory cytokines and immune cells or secondarily produced by alternatively-activated (M2) macrophages and regulatory T-cells induced by interactions with or uptake of exogenously-administered MSCs. One of the most important growth factors, HGF reduces kidney fibrosis by blocking tubular epithelial cell differentiation and inhibiting intra-renal expression of MCP-1 and macrophage infiltration. (70, 71) Other key mediators associated with the direct and induced paracrine effects of MSCs in DKD include IDO, a potent immunomodulatory enzyme; PGE2, a likely mediator of regulatory T cell differentiation and IL-10, an anti-inflammatory cytokine released by macrophages following phagocytosis of apoptotic MSCs. (64, 72, 73) The many observations from experimental models of DM and DKD that key soluble and released factors mediate the disease modulatory effects of MSCs have also stimulated interest in the use of MSC-derived conditioned medium and EVs as alternative DMRT. (74, 75) Despite the focus on paracrine mechanisms in many pre-clinical studies, however, it remains unclear whether soluble factors released by MSCs following systemic delivery can explain all of the beneficial effects reported in experimental DKD. Specifically, the transient survival of intravenously administered MSCs and reports in other disease models of therapeutic effects mediated by apoptotic or heat-inactivated MSCs suggest the existence of other mechanisms. (47, 48, 72, 76) Although transmigration and prolonged engraftment of a minority of administered cells to the kidneys remains theoretically possible, the phenomena of MSC apoptosis and
efferocytosis by macrophages(48) and MSC-induced expansion of T-reg(77) represent more compelling mechanisms by which their anti-inflammatory, pro-repair effects within the kidneys could be augmented and prolonged beyond the initial release of soluble mediators.

In addition to MSCs from various tissue sources, similar renal regenerative effects have been observed for a variety of other stem/progenitor-like cells and their secreted trophic factors or EVs.(36, 68) Primary cells derived from the kidney may also exert paracrine-mediated disease modulating effects in similar fashion to MSCs and are being actively pursued as potential DMRT. For example, selected renal cells (SRC), comprised of isolated tubular and aquaporin 2-positive collecting duct cells, have advanced to the clinical translation phase.(78) These primary cells induce tubular cell proliferation while attenuating TNF-α-induced NF-κB and TGF-β1-mediated PAI-1 signaling pathways that contribute to inflammation and fibrosis in experimental DKD.(79) Given the transient period in which exogenously administered cells reside in the diseased microenvironment, use of biomaterials such as hydrogels to enrich cell delivery and duration of action have been pursued.(13, 80) As discussed below, this has since been translated to a locally-delivered therapeutic strategy for DKD in which a gelatin-based hydrogel containing expanded autologous SRC is implanted into the kidneys.(78)

Despite numerous studies in experimental DKD, clinical translation of DMRT has been limited. In 2016, Packham et al. reported the results of a randomized, placebo-controlled, dose-escalation study which tested the safety and feasibility of intravenous infusion of allogeneic mesenchymal precursor cells (rexlemestorcel-L) in adults with type 2 DM and CKD stage 3/4. The cell infusions were well tolerated and trends in kidney function during a 12-week follow-up period favored stabilization or improvement in 20 cell-treated compared to 10 placebo-treated patients.(81) Other early-phase clinical trials are now investigating allogeneic bone-marrow derived MSCs (MDG; Italy, Ireland, United Kingdom, ClinicalTrials.gov NCT02585622), autologous adipose-derived MSCs (LJH, SMH; United States, NCT03840343), and allogeneic umbilical cord-derived MSCs (Japan, NCT04125329;
China NCT04216849) in DKD. As mentioned above, the therapeutic combination of primary kidney cells (SRC) in hydrogels (named Neo-Kidney Augment) is also being investigated as a DMRT for DKD in phase I and II clinical trials (NCT02008851, NCT03270956, NCT02836574). Of note, a report of the phase I trial involving laparoscopically-assisted intracortical implantation of SRC in 7 male patients with T2DM and CKD stages 3/4 indicated an unacceptable number of post-procedural complications prompting changes in implantation methodology. Nonetheless, renal function and urine ACR remained relatively stable for 12 months while eGFR tended to decline from months 12-24 after SRC administration.(78) Though also promising, injection of DRMT-derived EVs is not yet underway in human DKD studies.

**Kidney Transplantation**

Beyond the “holy grail” of donor-specific tolerance, steady advances in understanding pathological processes that underlie the common causes of early and late renal allograft failure have revealed other important new therapeutic targets that are not adequately addressed by conventional immunosuppressive drugs and clinical practices.(82) These include inflammatory and metabolic pathways that mediate donor organ injury prior to and early after transplantation, immunological processes that drive the formation of donor-specific antibodies (DSA) and anti-donor memory T cells, effector mechanisms responsible for subsequent acute or chronic immune-mediated rejection and maladaptive cellular processes such as fibrosis and senescence. Against this backdrop, the potential for DMRT such as MSCs and regulatory immune cells to address some or all of these major unmet needs for improved long-term KTx survival is being robustly pursued. In the following paragraphs and illustrated in **Figure 5**, we provide overviews of recent progress in the translation of these DMRT to the field of kidney transplantation and how they may address key mechanisms of graft injury.

*Mesenchymal stem/stromal cells:* Extensive pre-clinical evidence that MSCs modulate harmful anti-donor immune responses and maladaptive inflammation associated with
allogeneic organ transplants and may promote immune tolerance has accumulated over the past 18 years.(83) In 2012, Tan et al. reported the results of a Phase 2 clinical trial in which 104 living related donor KTx recipients received a novel induction regimen consisting of two intravenous infusions of autologous bone marrow-derived MSCs at the time of transplantation and 2 weeks later followed by maintenance therapy with conventional- or low-dose cyclosporine. For MSC-induced recipients, early recovery of renal function and frequency of acute rejection and opportunistic infection during the first post-transplant year were comparable or superior to those of a control group induced with anti-IL2R antibody followed by conventional-dose cyclosporine.(84) Although the trial provided an encouraging demonstration of the safety and potential efficacy of peri-transplant MSC infusions, the lack of mechanistic studies and of a measurable indicator of the in vivo activities of the infused cells precluded any immediate progress toward wider clinical practice. For this reason, several other centers have focussed on evaluating both autologous and allogeneic MSC therapies in smaller numbers of KTx recipients along with longitudinal immunological and, in some cases, histological monitoring of the grafts. Details of the designs, major outcomes and documented immunological consequences of MSC administration to KTx recipients in such early-phase trials carried out to date have been summarized and expertly reviewed by Podestà et al.(83) Results from one further phase 1 trial have also been very recently reported.(85) In addition to determining safety profiles, these trials have begun to address whether autologous or allogeneic MSC infusions can: (a) promote T-reg and/or donor-specific T cell hypo-responsiveness,(86-88) (b) reverse or stabilize subclinical tubulointerstitial inflammation and fibrosis/tubular atrophy(89) and (c) allow for early or delayed reduction (or even eventual withdrawal) of calcineurin inhibitor-based immunosuppression.(85, 90, 91) A further interesting question, currently being addressed in pre-clinical studies(92-94) as well as an early-phase clinical trial (NCT04388761), is whether ex vivo perfusion of kidneys procured for transplantation with MSCs can modulate ischemic tissue injury and ameliorate subsequent delayed graft function.
Taken together, the trial reports to date support the conclusion that intravenous or intra-renal infusion of MSCs in KTx recipients is safe and associated with comparable early-to-mid-term patient/graft outcomes and potentially superior renal function compared to conventional therapeutic regimens. Where examined, they also provide evidence that pre- or early post-transplant MSC infusions may be associated with favorable immunological effects such as increases in circulating T-reg or T-reg/effector T cell ratios. Nonetheless, it should be acknowledged that overall patient numbers remain small and the possibility of more subtle adverse effects such as localized pro-inflammatory response, reduced anti-viral immunity or, in the case of allogeneic MSCs, stimulation of anti-HLA antibodies should be carefully addressed by larger trials and longer follow-up.(83) Development of clinically-applicable assays of potency and in vivo effects in the context of kidney transplantation will also likely be required for optimal translation of MSCs and other DMRT into routine clinical practice. For example, for the clinical target of acute graft versus host disease following allogeneic hematopoietic stem cell transplantation, Cheung et al. recently demonstrated that an increase in serum PGE2 one to four days following MSC infusion distinguished patients with clinical response to cell therapy from non-responders – a finding consistent with in vitro cellular assays and mechanistic animal model studies carried out by the same group.(48, 95)

Regulatory immune cells: Since the recognition that FoxP3+ T-reg are essential for maintaining peripheral immune tolerance to auto-antigens and non-threatening foreign antigens, the concept that treatment with ex vivo-expanded regulatory immune cells could prevent allogeneic transplant rejection and foster donor-specific tolerance in organ allograft recipients has been energetically pursued.(96, 97) In addition to T cells, it has also become clear that other major types of immune effector cell, including macrophages, dendritic cells and B cells incorporate subpopulations or alternative functional states that mediate counter-regulatory/suppressive effects and may be amenable to clinical exploitation.(97) In keeping with the concept of DMRT, the potential therapeutic actions of regulatory immune cell therapies can be broadly viewed as modulating (as opposed to blocking) the interactions
between donor-derived (allo)-antigens or pro-inflammatory stimuli and recipient immune effectors to prevent or reverse acute and chronic organ allograft injury. Based on evidence from almost two decades of basic and pre-clinical research, early phase clinical trials of ex-vivo expanded autologous T-reg have been recently completed in kidney as well as liver transplant recipients.\(^{(8, 96, 98-101)}\) Those carried out in KTx recipients have, to date, consistently demonstrated safety in combination with various conventional immunosuppressive regimens as well as preliminary evidence for persistence of infused T-reg in the blood for 1-3 months\(^{(99)}\) and of an increase in total circulating T-reg numbers for at least 12 months after administration.\(^{(98)}\) Ongoing early phase trials and planned phase 2 trials are likely to further clarify whether polyclonal, autologous T-reg therapies robustly modulate post-transplant immune responses toward donor-specific tolerance.\(^{(96)}\) Other regulatory immune cell types – specifically regulatory macrophages (M-reg) and tolerogenic dendritic cells (tol-DC) - have also been developed to the point of early phase clinical trials in KTx recipients.\(^{(102, 103)}\) Very recently, a report of the post-transplant outcomes and immunological profiling results for living donor KTx recipients enrolled into a suite of early phase regulatory cell therapy trials has been published by the ONE Study consortium.\(^{(100)}\) In this unique study, the observed results for 38 living donor KTx recipients receiving one of four different T-reg, one M-reg or one tol-DC therapeutic product in combination with a tapered conventional immunosuppressive drug regimen were collated and compared with results for a group of 66 recipients that was managed by a standard-of-care regimen across eight sites in Europe and the US. Although no conclusions about the pro-tolerogenic efficacy for any single regulatory cell therapy can be made from this report, the authors convincingly show a good safety profile for such therapies in KTx recipients. In addition, the combined cell therapy cohort had no increase in graft rejection or survival, experienced a strikingly reduced rate of viral infections and appeared to revert to a more favorable peripheral blood immune cell profile compared to conventionally-treated recipients.\(^{(100)}\) Subsequently, Roemhild et al. published a very detailed report of the ONEnTreg13 trial - a component of the ONE Study. In this phase I/IIa trial, autologous, ex vivo-expanded natural (n)T-reg were
administered 7 days after transplantation to 11 living donor KTx recipients at Charité Universitätsmedizin, Berlin, Germany with results compared to those of 9 previously-transplant patients managed by the standard-of-care regimen at the same centre. These results demonstrated excellent safety and 3-year graft outcomes for the nTreg-treated KTx recipients as well as successful tapering of immunosuppression to tacrolimus monotherapy in 8/11 subjects and evidence of oligoclonal (presumably allo-antigen-driven) expansion of nTeg at 60 weeks post-transplant. (101) Finally, in another very recent early phase trial report, Morath et al. describe favorable safety and graft outcomes following pre-transplant administration of donor-derived modified immune cell (mitomycin C-treated PBMC) infusions to 10 living donor KTx recipients. Post-transplant immunological studies also provided evidence for prolonged donor-specific T cell hypo-responsiveness and increased numbers of circulating IL-10-producing regulatory B cells (which have been associated with immune tolerance). (104)

Overall, recently reported and ongoing early phase clinical trials of several cell-based DMRT in the area of kidney transplantation provide important reassurances regarding the safety and feasibility of such therapies both prior to and at early or later times after transplantation. The generally favorable short-to-mid-term patient and graft outcomes and promising immune profiling and histological studies should be interpreted with caution in the absence of larger, randomised controlled trials. Nonetheless, cohesion among some of the observations made in patients participating in these trials and the growing scientific knowledge regarding immunological tolerance and of the pathobiology of KTx complications should provide a strong impetus for further progress. As illustrated in Figure 5, individual DMRT have the potential to target specific clinical and immunological challenges associated with current limitations to the long-term health of kidney KTx recipients.

**Autologous versus Allogeneic Cell Therapies: The Influence of Patient-specific Factors**
As we move toward clinical application of DMRT, optimization of cell product remains vital to successful translation of preclinical findings. Allogeneic cell-based products offer a readily available “off-the-shelf” treatment option. Yet, patient-derived (autologous) cells may be preferable for individualized therapy or for repeated dosing, given the lower risk for allosensitization. In this regard, the influence of patient- or disease-specific factors on the growth and functionality of culture-expanded stromal and other primary cell therapies represents a key research area – particularly in settings of high relevance to kidney disease such as older age, DM, vascular disease and reduced renal function. In DM and kidney disease, oxidative stress, autophagy, and cellular senescence induce dysfunction of autologous cells. In metabolic syndrome and DM, stem cell mobilization and therapeutic effect are diminished. In older individuals with atherosclerotic RVD, we identified altered MSC functional capacity (migration, angiogenesis) and increased cellular senescence burden compared to controls. Despite this, our clinical trial results described above in RVD, confirm that intra-renal administration of autologous MSC was associated with improved RBF and preserved GFR. Similarly, in MSCs harvested from adults with DKD and healthy controls, we have observed transcriptome alterations and reduced in vitro MSC migration but preserved or increased immunomodulatory and renal reparative activities in vitro (LJH, under review). In ESRD and KTx recipients, autologous MSCs have been shown to undergo comparable culture expansion characteristics to those from individuals with normal kidney function and to maintain the capacity to inhibit anti-donor HLA immune response compared to control MSCs.

Despite these encouraging results, counteracting biological processes that potentially limit the regenerative functions of manufactured cell products may prove to be important for maximizing the benefits of autologous cell therapies. Recent developments in the understanding of cellular senescence may offer exciting opportunities for the application of DMRT to kidney disease. Premature senescence reduces MSC replicative capacity and limits cell expansion in manufacturing protocols. The abundance of senescent cells also
fuels proinflammatory pathways in the pathogenesis of disease processes such as DM and DKD.(108, 109) Furthermore, the microenvironmental stressors of kidney diseases (uremia, hyperglycemia, kidney aging, renin-angiotensin-aldosterone system alteration, oxidative stress, inflammation) contribute to senescent cell accumulation, potentially diminishing endogenous and exogenous MSC regenerative capacity.(105, 110) Emerging therapeutic strategies offer the possibility of modulating the microenvironments from which primary stromal cells are extracted through senescent cell clearance in vivo. In a pilot clinical trial, we recently observed that a 3-day oral senolytic regimen of dasatanib and quercetin can diminish senescent cell abundance in adipose and epithelial tissue and improve MSC proliferation in subjects with DKD.(111) We and others are also pursuing other preconditioning methods to optimize autologous MSC functionality. Interventions such as exposure to hypoxia or melatonin during culture expansion may enhance the pro-repair properties of MSCs.(32, 112) Taken together, these insights suggest that further integration of in vivo or ex vivo conditioning regimens could enhance the success of autologous (and perhaps also allogeneic) cell-based DMRT in kidney disease.

Conclusions and Future Directions

As we have reviewed here, extensive pre-clinical research has greatly increased our understanding of the in vivo distribution, longevity and mechanisms of action of MSCs and other potential regenerative therapies in the setting of kidney diseases. These insights have extended the rationale for regenerative therapies beyond the initial concept of engraftment and differentiation into functional tissues to include modulatory effects of, typically, short-lived cells, vesicles or biomaterials that ameliorate disease processes through complex, paracrine interactions with host cells and tissues which promote inherent mechanisms of repair and regeneration. Well-conducted small and large animal model studies have been essential to determining the optimal parameters for clinical application of MSCs and other DMRT to kidney diseases – including the route of delivery and localization following administration, the key responder compartments and cell responses within the kidney, and
the source, timing of release and activity of the most important soluble mediators. In keeping with the literature reviewed in this article, we believe that these critical parameters must be defined for each specific disease and therapy. Conversely, lack of concordance between animal model research and human clinical application of regenerative therapies continues to be a major challenge to the field. For the four distinct renal disease areas we have focussed on, translation of DMRT into clinical nephrology practice remains at an early stage. Indeed, of the translational strategies we review here, only MSC therapy for RVD and T-reg therapy in KTx could be said to have shown preliminary (early-phase) clinical trial evidence of superior efficacy compared to conventional pharmacotherapy and interventional procedures. Nonetheless, research in this area has generated a wealth of novel scientific insight and a growing number of informative clinical trial experiences with MSC- and regulatory immune cell-based investigational medicinal products. Reassuringly, the safety profiles for such cell therapies in patients with kidney diseases and KTx enrolled into clinical trials has, thus far, proven to be very good. For some of these clinical applications, preliminary signals of in vivo disease modulation have also emerged(26, 33, 84, 86, 88, 100, 101, 104) while others have lacked evidence of efficacy.(58)

In considering how future clinical impact could be maximized for cell-based DMRT that have undergone early-phase clinical trials in patients with kidney disease, a number of critical missing elements should be highlighted: (a) Development of disease-specific assays to quantify potency of and patient response to DMRT in order to account for complexities such as inter-individual heterogeneity and changes in cell functionality that occur during ex vivo expansion. (b) Definition of optimal dosing, distribution and frequency of administration of DMRT for specific clinical targets. (c) Consensus on the influence of cryopreservation which may negatively impact the consistency of DMRT therapeutic effects.(6, 7, 113) (d) Increased understanding of relative clinical efficacy of autologous and allogeneic cell therapies for specific patient groups. (e) Innovations in manufacturing procedures that will eventually allow for cost-effective delivery of DMRT to large numbers of patients. As is clear from Figure 1,
other DMRT for which pre-clinical evidence bases and technological developments are building await definitive clinical translation.(13, 36, 114) Increasingly, cross-discipline research and innovation has brought the potential for combinatorial advanced therapies to the forefront of translational initiatives in regenerative medicine. Incorporation of gene editing and biomaterials science holds future promise for stabilizing and enhancing the key mechanisms of action for cellular therapies identified from pre-clinical studies or patient profiling in early phase clinical trials. Similarly, clever use of combined pharmacotherapy and DMRT is likely to be critical for optimizing and broadening the clinical applications of regenerative medicine. For example, patient conditioning through the co-administration of anti-senescence ("senolytic") agents to enhance MSC survival and anti-inflammatory/immune regulatory responses.(31, 111)

Viewed through the lens of four distinct areas of clinical nephrology practice, we conclude that the potential for our patients to substantially benefit from DMRT within the next decade is high and will be driven by a spirit of “joined up thinking” among basic scientists, biomedical engineers, technology innovators, clinical triallists, clinicians and funding and regulatory bodies.
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Figure Legends

Figure 1: Progress toward clinical translation of five categories of disease modulating regenerative therapies for four different areas of nephrology practice: The figure summarises current translational status of mesenchymal stromal/stem cells, regulatory immune cells, other adult progenitor cells, extracellular vesicles and hydrogel/biomaterials as potential DMRT for renovascular disease (RVD), sepsis-associated AKI (SA-AKI), diabetic kidney disease (DKD) and kidney transplantation (KTx). * Broken line indicates that clinical trials of MSCs have been reported in sepsis including some patients with SA-AKI but not with kidney function as a primary outcome. * Figure created using Biorender.com.

Figure 2: Potential strategy for enhancing MSC therapeutic potency in hypertensive kidney disease: As illustrated, chronic hypertension, renovascular disease, and ischemic nephropathy lead to stromal cell dysfunction which is associated with limited production of paracrine regenerative factors (released extracellular vesicles (EVs) and soluble mediators) by patient-derived, culture-expanded mesenchymal stromal cells (MSC). Culture under low oxygen tension (Hypoxic Pre-conditioning) may restore the production of EVs and soluble mediators resulting in enhanced paracrine regenerative activity and increased potential for disease modulation following localized or systemic delivery of autologous MSCs. * Figure created using Biorender.com.

Figure 3: Elements of the potential therapeutic effects of mesenchymal stromal cell therapy in sepsis-associated AKI: As illustrated, intravenous administration of MSCs in the setting of sepsis-associated AKI results in MSC trapping in the lungs where complex interactions (cross-talk and efferocytosis) with resident immune cells [mononuclear phagocytes (macrophages) and lymphocytes] result in beneficial localized effects within the alveolar spaces as well as systemic effects (LPS neutralization, secretion of anti-inflammatory
factors, enhanced phagocytosis) with potential to promote resolution of inflammation, disrupted vascular integrity and increased cell death in the kidneys. Improved cardiorespiratory function as a result of MSC local and systemic effects may provide further indirect effects to more effectively resolve sepsis-associated AKI. Abbreviations: AKI – acute kidney injury; MSC = mesenchymal stromal cells; LPS = lipopolysaccharide; IL-10 = interleukin 10; PGE2 = prostaglandin E2; VEGF = vascular endothelial growth factor, KGF = keratinocyte growth factor; HGF = hepatocyte growth factor. *Figure created using Biorender.com.*

**Figure 4: Multiple potential therapeutic effects of systemically-administered mesenchymal stromal cells in diabetic kidney disease:** Extensive pre-clinical and limited clinical trial data indicate that MSCs (*Centre*) may exert both extra-renal and intra-renal modulatory effects through a range of key mediators following intravenous administration in diabetes and DKD. *Upper left:* Extra-renal effects which diminish adipose tissue inflammation, enhance insulin sensitivity and preserve islet function can stabilize or reverse the course of DKD by improving glycemic control and reducing systemic inflammation and oxidative stress. *Lower right:* Intra-renal effects key MSC-generated and -induced mediators have been shown experimentally to modulate multiple aspects of DKD pathophysiology within the glomerulus, the tubulointerstitial compartment and the microvasculature. *Figure created using Biorender.com.*

**Figure 5: Disease modulating regenerative therapies in kidney transplantation:** Upper Panel: Diverse types of modulatory cellular therapies that have been the subject of early phase clinical trials in kidney transplant recipients along with their potential sites of action and target cells. Middle Panel: Four major mechanistic goals of DMRT applied to kidney transplantation with illustration of the most relevant cellular therapies for each along with their predicted sites of action and most significant cell targets for each (based on pre-clinical
studies and a profiling/monitoring analyses of clinical trial subjects). Lower Panel: Significant clinical benefits that represent the most immediate goals for the clinical translation of DMRT in kidney transplantation. Abbreviations: DMRT = disease modulating regenerative therapy; MSC = mesenchymal stromal cells; T-reg = regulatory T cell; M-reg = regulatory macrophages; Tol-DC = tolerogenic dendritic cells. Figure created using Biorender.com.
Figure 2

Hypertension

Renovascular Disease

Ischemic Nephropathy

Stromal Cell Dysfunction

Disease Modulation

Limited MSC Paracrine Regenerative Factors

Hypoxic Pre-conditioning Inflammatory Stimuli

Enhanced MSC Paracrine Regenerative Factors
Sepsis + AKI

Increased bacterial, cellular and fluid clearance; resolution of inflammation

MSC/Immune Cell Cross-talk and Efferocytosis

Neutralization of LPS; Increased secretion of IL-10, PGE2, VEGF, KGF, HGF; Enhanced macrophage phagocytosis;

Reduced pro-inflammatory cytokines; Improved vascular permeability; Enhanced clearance of apoptotic cells

Renal functional improvement secondary to improved cardiorespiratory function

AKI Resolution
Figure 5

- **DMRT**
  - MSC
  - T-reg
  - M-reg
  - Tol-DC

- **Site of Action**
  - Systemic
  - Local

- **Target Cells**
  - Naive Lymphocytes
  - Activated Lymphocytes
  - Myeloid Cells
  - Dendritic Cells
  - Fibroblasts
  - Epithelial Cells

- **Promote Donor-specific Immune Tolerance**
- **Modulate Pre-existing Anti-donor Immune Responses**
- **Promote Epithelial Repair and Regeneration**
- **Suppress Pro-fibrotic Inflammation**

- **Reduced ischemic injury / Delayed Graft Function**
- **Increased maximum glomerular filtration rate**
- **Reduced burden of immunosuppressive medications**
- **Stabilization of declining transplant function**