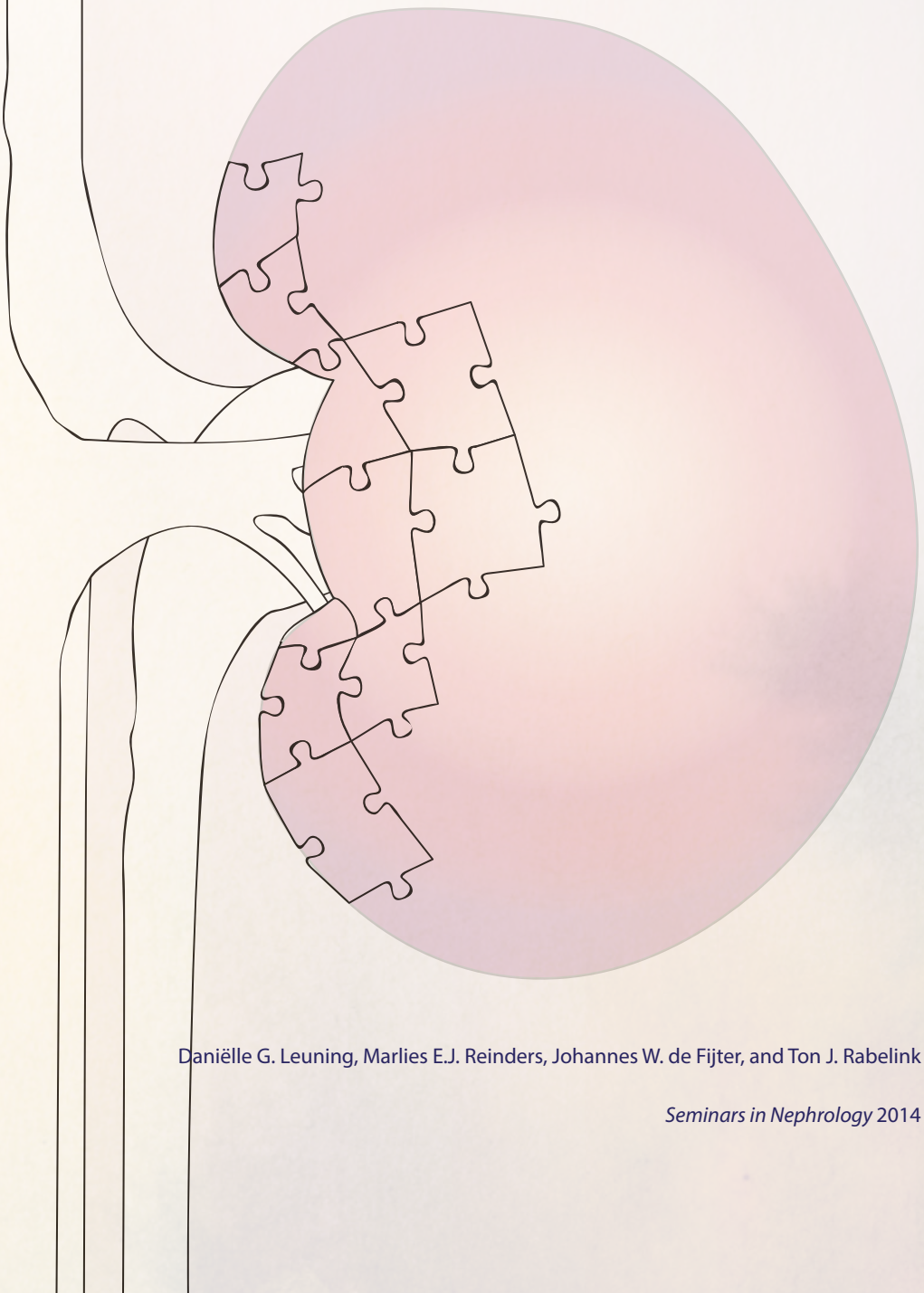
Development of novel strategies to regenerate the human kidney

Leuning, D.G.

Chapter 2

Clinical translation of multipotent mesenchymal stromal cells in transplantation



Daniëlle G. Leuning, Marlies E.J. Reinders, Johannes W. de Fijter, and Ton J. Rabelink

Seminars in Nephrology 2014

Abstract

The prevalence of chronic kidney disease and end stage renal disease (ESRD) is increasing each year and currently the best therapeutic option for ESRD patients is kidney transplantation. However, although short-term graft outcomes after transplantation have improved substantially due to new and more potent immunosuppressive drugs, the long term survival has hardly changed. This is most likely caused by a combination of non-immunological side effects and sustained alloreactivity to the graft resulting in fibrosis. In addition, current immunosuppressive drugs have side effects, including nephrotoxicity, infections and malignancies that compromise long-term outcomes. Consequently, there is a strong interest in immunosuppressive therapies that maintain efficacy, while reducing side effects. As mesenchymal stromal cells (MSCs) have potent anti-inflammatory and anti-fibrotic properties, these cells are of particular interest as new candidates in transplant recipients. MSCs might play roles in the treatment of allograft rejection and fibrosis and in calcineurin minimization and induction protocols. In the present review we discuss both preclinical as well as clinical evidence of their therapeutic potential in kidney transplantation. In addition, challenges and obstacles for clinical translation will be discussed.

Introduction

Chronic kidney disease (CKD) is a common disease in the western population as at least 8% of this population has a degree of CKD, placing them at a moderate to high risk to develop kidney failure[1]. This figure is increasing each year due to an aging population and an increase in the prevalence of chronic (reno) vascular morbidity and diabetes. If the present trend continues, the number of people with CKD will double over the next decade.

Although there are strategies to slow down the progression to end stage renal disease (ESRD), there are currently no therapies to cure CKD, therefore approximately 5% of patients with a diminished kidney function will progress into ESRD with the need of renal replacement therapy[2]. Currently the best therapy for these patients is kidney transplantation as this improves the life expectancy and quality of life of ESRD patients.

In transplanted patients, the graft survival rate has increased in the last decades to over 90%, mainly due to the reduction of acute rejection within the first year after transplantation. However, long term graft survival has remained unaltered over the last two decades [3]. Both immunologic and non-immunologic factors contribute to the development of fibrosis in the allograft, including ischemia reperfusion injury (IRI), ineffectively or untreated clinical and subclinical rejection, superimposed calcineurin inhibitor nephrotoxicity and exacerbating pre-existing donor disease. Moreover, long-term systemic immune suppression is increasingly recognized for its numerous side effects, of which infections and malignancies are the most important. There is therefore a need for novel treatment strategies to improve both the immunological acceptance of the graft as well as to prevent fibrosis and foster the regenerative capacity of the transplanted kidney. Mesenchymal stromal cell (MSC) based therapy shows to be a promising candidate for both situations as their properties may influence inflammation and fibrosis.

MSC characteristics

MSCs represent a minor fraction of the bone marrow (0.01-0.001%). They are typically located perivascularly where they control the vascular bone marrow niche and through differentiation into osteoblast give rise to the niche for long term repopulating stem cells [4]. They are easily isolated from the bone marrow as they adhere to plastic and can substantially proliferate and expand in culture [5,6]. Unfortunately there is no specific MSC marker and therefore a set of phenotypic and functional criteria were proposed by the International Society of Cellular Therapy (ISCT) including their plastic adherence, trilineage differentiation potential and the expression of the stromal markers CD73, CD90 and CD105 while being negative for the hematopoetic markers

CD14, CD34 and CD45 [7]. While several attempts have been made to select a more homogeneous MSC population by using surface markers such as e.g. STRO-1, there is so far no unique marker that allows direct MSC isolation and selection. This may be due to the fact that MSCs actively interact with surrounding cells through microvesicles and exchange of cell components [8].

In addition to the bone marrow a population of perivascular localized MSCs can be found in several, if not all, organs, including the kidney [9,10]. In the mouse, kidney derived MSCs showed similar marker expression and differentiation potential compared to bone marrow (bm)MSCs; however, when looking at mRNA expression profile and in vivo differentiation potential, these cells appeared strikingly different [10]. These differences may be caused by tissue specific imprinting during embryogenesis or local cues. This makes kidney (and other organ) derived MSCs of particular interest as a new source for renal repair. Bruno et al. showed that kidney derived MSCs can be isolated from human glomeruli as well. Whether there are differences between these MSCs and bmMSCs and whether these differences lead to improved renal repair mechanism needs to be further elucidated [11].

The ubiquitous presence of MSCs in tissues probably reflects to a large extend the embryological development of the structure of tissues and has also initiated the isolation and expansion of MSCs from other tissues for clinical use. These include adipose tissue, umbilical cord and dental pulp [12-14]. We will discuss later the advantages and drawbacks of the use of these sources as compared to the use of bmMSCs.

Immunomodulatory and reparative functions of MSCs

Due to their perivascular location, MSCs can closely interact with several cell types including endothelial cells, resident macrophages, dendritic cells (DCs) and recruited inflammatory cells (figure 1).

The immunomodulatory potential of MSCs is the function most extensively studied. MSCs interact with several key players of both the innate as the adaptive immune system as extensively reviewed elsewhere [15,16]. In short, MSCs are able to suppress T-cell proliferation and favor the formation of regulatory T-cells (Tregs). This is in human MSCs to large extent caused by the secretion of soluble factors including indoleamine 2,3-dioxygenase (IDO), transforming growth factor β (TGF-β) and prostaglandin E2 (PGE2) [17-19]. With respect to B-cells the effects of MSC therapy is more variable and sometimes even contradictory. In the B-cell driven disease systemic lupus erythematosus (SLE), for example, some studies have shown a beneficial effect of MSC

therapy on kidney function, complement depositions and the levels of circulating double stranded DNA [16,20], while others did not see an effect on kidney function and survival [21] or even showed a negative effect resulting in increased disease activity [16,22].

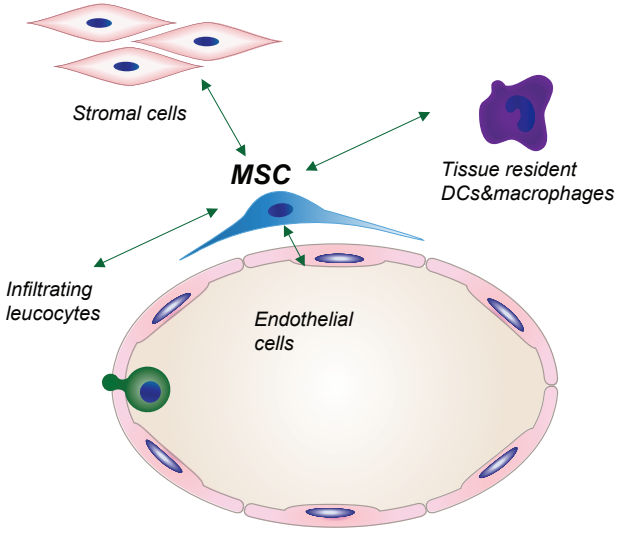


Figure 1. Central location of MSCs. MSCs have, due to their perivascular location, interactions with endothelial cells, stromal cells, tissue resident DCs, macrophages and infiltrating leucocytes. Because of all these interactions they are central in tissue homeostasis.

MSCs do not only interact with the adaptive immune system but also with components of the innate immune response. MSCs are, for example, able to interfere with DC migration, maturation and antigen presentation via secretion of Interleukin (IL)-6, M-CSF, PGE2 and IL-10 [16]. In addition, MSCs have also been shown to modulate natural killer cell (NK cell) responses [23]. These effects of MSCs may recapitulate their function in the bone marrow niches, where one of their main functions is to maintain an inflammatory milieu that allows for progenitor cell maturation and release of blood cells into the circulation [24]. It is also important to realize that part of the regenerative properties of MSCs may depend upon their ability to polarize the immune system into a reparative phenotype. For example, M2 macrophages have been identified as tissue reparative myeloid cells in the kidney by producing a cytokine environment that supports tubular repair and proliferation rather than inflammation [25]. In agreement, genetic or pharmacological blockade of CSF1 decreases M2 polarization and subsequently inhibits recovery from acute kidney injury [26].

It is becoming, however, increasingly clear that MSCs do not always display this anti-inflammatory phenotype. It turns out that in fact MSCs, like many cells of the immune system, can be polarized both into an anti-inflammatory (MSC2) phenotype or a pro-inflammatory (MSC1) phenotype (for review see [17]). The immunosuppressive phenotype of MSCs depends on exposure to interferon-gamma (IFNy), which in the presence of other inflammatory cytokines (such as TNFa, IL-1a or IL-1b) induces nitric oxide synthase (iNOS) in the case of rodent MSCs or IDO in the case of human MSCs. In the absence of IFNy or iNOS/IDO, the MSCs can actually turn into immune competent cells [27]. Obviously this imposes an important challenge to the clinical administration of MSCs. Dependent upon the timing and the local milieu where they home to, they may lose their immunomodulatory properties.

MSCs also interact directly with endothelial cells via the production of paracrine factors (including vascular endothelial growth factor (VEGF) and angiopoietin 1 (ANG-1)) with the aim to stabilize and maintain the microvascular architecture and thus tissue perfusion [28,29]. Not only paracrine mechanisms but also cell-cell contacts are of importance for vessel stabilization. As an example, knock down of the α6β1 integrin receptor in MSCs leads to decreased capillary sprouting and failure of vessels to associate with nascent vessels [30]. Of note, perivascular MSCs may probably, beyond the point of regeneration, also contribute to fibrosis. Indeed, Humphreys et al showed using lineage tracing studies of FoxD1^{+} pericytes, that perivascular stromal cells can transform into myofibroblasts upon severe kidney injury, and contribute to renal fibrosis as a last resort repair mechanism [31].

In summary, MSCs are ubiquitous perivascular stromal cells that regulate tissue homeostasis. When primed by the adaptive immune system they can induce polarization of the immune system towards down regulation of inflammation and vascular stabilization. However, it is important to realize when applying these cells as a potential therapy, that without the proper priming components of the innate immune system MSCs can turn into cells that can activate the immune system and drive fibrotic repair.

MSC therapy in experimental models of kidney disease and transplantation

In several experimental models of kidney disease and transplantation, MSC treatment enhanced tissue repair and reduced fibrosis as reviewed in a meta-analysis elsewhere [32]. Although there is a large variation in disease models, source (human/animal MSCs), amount, timing and administration route of MSCs in these studies, in general there is a benefit of MSC therapy [32]. Interestingly, MSC infusion in a transplantation setting may not only reduce the inflammatory reaction but may also induce a state of allograft tolerance. In a sensitized murine kidney

transplantation model for instance, MSC infusion prior to transplantation resulted in long term graft survival, up to 60% for more than 2 months, while in control animals all grafts were rejected at 10 days post transplantation. There was a donor specific T-cell hypo responsiveness and an increase in Tregs in the mice who received the MSCs pre-transplantation. However, when MSCs were infused post-transplantation this effect was not seen and in fact, there was a decrease in kidney function after MSC infusion, indicating that the timing of infusion (and thus the environment) is of importance for MSC treatment [33]. In another murine kidney allograft model the soluble factor IDO was shown to be crucial for long term allograft survival [34].

It is thought that most of the aforementioned effects of MSCs are caused via paracrine mechanisms, including growth factors, microvesicles and other soluble factors such as IDO, vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) [35-39]. As demonstrated by Eggenhofer et al., i.v. infusion of murine bone marrow MSCs lead to accumulation in the lungs, where they disappear within 24h. This suggests that their local effects are endocrine or paracrine and transferred to other cell types, and this interaction may be crucial for the long term effects of MSCs [40].

MSCs in clinical trials: a bridge too far?

Bianco et al. criticized the ongoing clinical trials with MSCs as they argue that MSCs are not precisely enough characterized and are not *bona fide* stem cells [24]. They stated that the MSC population is too heterogeneous with the current characteristics as defined by the ISCT [41] and with these characteristics the clonogenicity and *in vivo* trilineage differentiation potential is not proven. Moreover, they argue that although there is enough evidence for MSCs as a skeletal stem cell, there is hardly any evidence for their immunomodulatory and tissue regenerative potential and because of these uncertainties they suggest more preclinical research before clinical trials.

However, for immunomodulatory functions MSCs may not need to fulfill the stem cell criteria. In fact, the word mesenchymal stem cell is misleading and the term mesenchymal stromal cell should be used instead. These mesenchymal stromal cells have a supportive role in all organs including tissue homeostasis and immune suppression, independent of their stem or progenitor potentials. And although the exact mechanisms of actions are still largely to be elucidated, both the preclinical and clinical data suggest immune suppression and a role of MSCs in kidney repair as summarized above. Therefore to our opinion, this should not be a limiting factor to perform clinical safety and feasibility studies. While animal studies may give some insights into safety and feasibility, the value of these studies is limited [42,43] probably due to the fact that genomic responses in mouse models poorly mimick human inflammatory diseases [44]. There is thus a need

for proper study designs with more standardized cell isolation, culture and infusion protocols and clinical trials should not only focus primarily on clinical outcomes but also on mechanisms of action and safety of MSC therapies.

Clinical studies using MSCs in kidney transplantion

In renal recipients, MSCs may be included in induction protocols, in the treatment of allograft rejection and fibrosis, and in calcineurin minimization protocols. The first phase I studies have been performed and are summarized in figure 2. Early studies focused on the role of MSCs in induction protocols. In a pilot study exploring safety and clinical feasibility 2 living-related kidney recipients were infused with bmMSCs 7 days after transplantation [45]. MSC infusion was shown to be feasible, allowing enlarging of Tregs in the peripheral blood and control of memory CD8+T cell function [45]. However, both patients showed a rise in serum creatinine 7-14 days after MSC infusion where a kidney biopsy excluded rejection but showed a focal inflammatory infiltrate. After treatment with methylprednisone there was a recovery of kidney function and 1 year-post transplantation the renal function of both patients was stable. As a nice example of 'from bedside to bench and back to bedside' development of new treatment strategies, the same group showed in a murine transplant model that MSC administration 1 day prior to kidney transplantation, opposed to post transplant administration, did not give graft dysfunction and in fact was able to induce tolerance [33]. Therefore in the second study, also including 2 living related kidney recipients, pre-transplant infusion of autologous MSCs were given. No engraftment syndrome or increase in serum creatinine levels were seen, although in one patient an acute cellular rejection occurred 2 weeks post-transplantation. This may, at least partly, be triggered by withholding basiliximab induction as the authors wanted to exclude an effect of basiliximab on Treg expansion. However, compared to previously described patients and controls there were no differences in Treg counts with or without basiliximab induction. Comparable to the 2 patients described in the previous study, in these two patients a subtle decrease in memory CD8 T cells was seen as well [46].

In a larger randomized study by Tan et al, the effect of autologous bmMSC infusion was studied as an alternative to anti-IL-2 receptor antibody for induction therapy in adults undergoing living-related donor kidney transplants [47]. MSC infusions were given on the day of transplantation just prior to surgery and at day 12, and were combined with triple therapy, both with standard dose calcineurin inhibition as well as with a lower dose. The rejection rate with MSC induction appeared lower (8 %) as compared to induction therapy with IL2 receptor blockade, which was relatively high at 20 %. Importantly, rejection rates increased from 6 to 12 months up to 17 %

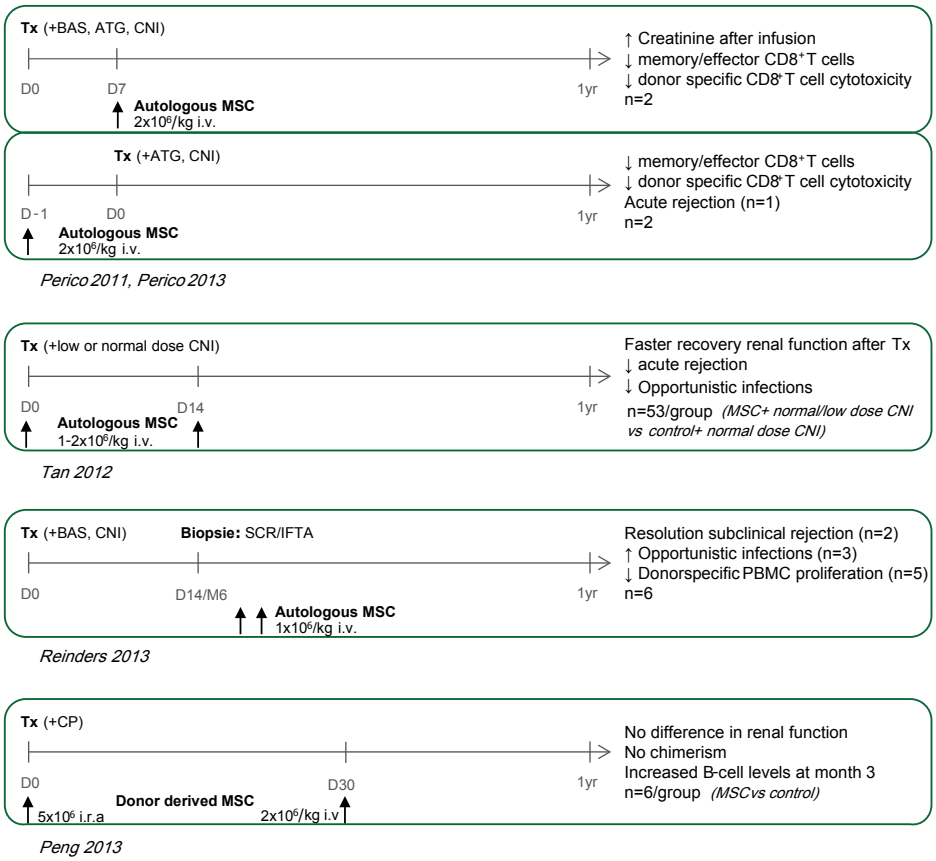


Figure 2. The set-up, timeline and results of the first clinical trials with MSC therapy for kidney transplantation. ↑: MSC infusion. Tx: transplantation, BAS: basiliximab, ATG: antithymocyte globulin, CNI: calcineurin inhibitor, CP: cyclophosphamide, i.v.: intravenous, i.r.a: intra renal artery

in the MSC arm, which may be related to the fact that initial depleting induction therapy was withheld. Moreover, the final rejection rates were substantially higher than what is usually observed in e.g. standard immune suppression regimes such as used in the Symphony study [48].

In our own phase I clinical trial, safety and feasibility of autologous bmMSC therapy was tested in subclinical rejection and/or in interstitial fibrosis and tubular atrophy (IFTA) [49]. Protocol biopsies were taken at 4 weeks and 6 months after transplantation and when these showed either subclinical rejection or IFTA, MSC therapy was given. MSC treatment showed to be feasible and there were no therapy related serious side effects. Two kidney recipients with a biopsy proven subclinical rejection showed a resolution of tubulitis without IFTA after MSC infusion.

Moreover, 3 out of in total six patients developed opportunistic infections after MSC therapy and in 5 patients a donor-specific down regulation of peripheral blood mononuclear cell (PBMC) proliferation was seen, all indicative of an immune suppressive effect of the MSCs in subclinical rejection and IFTA.

All studies listed above are performed with autologous, thus patient derived, MSCs. One study explored whether donor derived MSCs can also be used in transplant recipients. The investigators demonstrated that donor derived MSCs combined with low dose tacrolimus was safe and that patients who received MSCs had a similar renal function compared to controls with normal dose tacrolimus. There was no leucocyte chimerism after 3 months. There were almost no differences in leucocyte profiles and proliferation upon stimulation except an increase in B-cell levels in the MSC group compared to the control. Interestingly, in this study donorMSCs were first administered via the renal artery directly after reperfusion and secondly i.v. one month after transplantation [50]. Unfortunately the group size is too small to draw conclusions about both the method of administration and the potential advantages or disadvantages of donor derived compared to patient derived MSCs.

An interesting concept is to use MSCs as a means to minimize immune suppression. In our center, we have now embarked on a study to test the hypothesis whether MSCs in combination with a mTor inhibitor will facilitate tacrolimus withdrawal, reduce fibrosis and decrease the incidence of opportunistic infections compared to standard tacrolimus dose [NIH Gov NCT02057965]. Interestingly, in experimental studies the combination of mTor inhibitor and MSCs were shown to attenuate alloimmune responses and to promote allograft tolerance [51]. Indeed, combination therapy of MSCs and low-dose Rapamycin (Rapa) in a murine heart allograft model achieved long-term heart graft survival (>100 days) with normal histology. The treated recipients readily accepted donor skin grafts but rejected third-party skin grafts, indicating the establishment of tolerance. Tolerant recipients exhibited neither intragraft nor circulating antidonor antibodies, but demonstrated significantly high frequencies of both Tol-DCs and Tregs in the spleens [51].

Current MSC based trials (table 1) assessed mainly feasibility and safety issues. To document the induction of immune suppression after the treatment and to correlate the presence and magnitude to clinical outcomes, is of major importance to be able to monitor immune responses in MSC treated patients. This is crucial to help the clinician with critical issues including safety, dosage, frequency and timing of MSCs and might provide mechanistic insight. A first important step is the validation and standardization of the assays used for immune monitoring, which will facilitate fair and meaningful comparisons between trials. Interestingly, the One Study Consortium, which recently initiated a serie of clinical trials aimed at using different

cell therapies to promote tolerance to renal allografts, developed a robust immune monitoring strategy including procedures for whole blood leukocyte subset profiling by flow cytometry. Local performance and central analysis of this panel yielded acceptable variability in a standardized assay at multiple international sites and panels and procedures might be adopted as a standardized method in monitoring patients in clinical trials [52].

Challenges and obstacles in clinical studies that use MSC

If all the preclinical and clinical data are taken together, MSC therapy in kidney transplantation appears promising. There are however important considerations and concerns that need to be addressed. There is, for example, little known about the best source, timing, dosage, route of administration and frequency of cell administration. Besides, there is a need for more information regarding the safety concerns when using MSCs as cell therapy. In addition, there is discussion about the best early study design. These important issues will be described below.

1] Culture conditions for MSC isolation and expansion

Since the bone marrow contains only 0.001-0.01% primary MSCs, significant ex vivo amplification and culture is needed. There is a large variation in culture and isolation techniques of MSCs used in the clinical trials so far. Although most research have been performed with MSCs isolated and expanded on tissue plastic, the culture and cryopreservation protocols differ to large extent as reviewed extensively in previous reports[53]. For example, MSCs are mostly cultured in 5% platelet lysates while some use 10% fetal calf serum. MSCs grown in platelet lysate based medium in general grow faster although there are large variations when platelets from different donors are used [54]. The use of standardized media and culture conditions is required to minimize variability and increase reproducibility.

2] Sources of MSCs

Most clinical studies up till now have studied the effect of bmMSCs. Another source of MSCs in clinical studies is the adipose tissue. Adipose tissue derived MSCs (ASCs) exhibit at first glance the same characteristics and immunomodulatory potential compared to bmMSCs. In experimental models of solid organ transplantation both bmMSCs and ASCs could inhibit rejection and/ or increase graft survival [33,55-58]. We have recently made a direct comparison between both cell types in a humanized skin allograft rejection model and found that both human bmMSCs and human ASCs were effective in inhibiting skin allograft rejection. Local administration of bmMSCs as well as ASCs reduced inflammation by inhibiting the recruitment of T cells and decreasing IFNγ, TNFα, IL-6 and IL-1β expression in the skin grafts[59].

Table 1. Currently registered trials for MSC therapy in kidney transplantation and kidney disease

Trial	NCT#	Sponsor	Study protocol	# pts.	Status
MSC after renal or liver transplantation	01429038	University Hospital of Liege, Belgium	Allogenic bmMSCs 3 days post-Tx	40	recruiting
Autologous MSCs in combination with Everolimus in Renal Recipients to preserve renal function and structure	02057965	Leiden University Medical Center, The Netherlands	2 doses of autologous bmMSCs (1-2x10^6mill. per/kg body weight)	70	not yet recruiting
MSC transplantation in the treatment of CAN	00659620	Fuzhou general hospital, China	Unknown	20	unknown
MSC for occlusive disease of the kidney	01840540	Mayo Clinic, USA	Arterial infusion autologous ASCs	15	recruiting
MSC in cisplatin induced acuted renal failure in patients with solid organ cancers	01275612	Mario Negri Institute, Italy	Allogeneic MSC i.v. Dose escalating	9	recruiting
Allogeneic multipotent stromal cell treatment for acute kidney injury following cardiac surgery	00733876	AlloCure, USA	Allogeneic MSC	15	Active, not recruiting
Phase II study of human umbilical cord derived MSC for the treatment of SLE (hUC-MSC-SLE)	01539902	Cytomed&Beike, China	ucMSC	25	recruiting
Effect of MSC transplantation for lupus nephritis	00659217	Fuzhou general hospital, China	Autologous MSCs compared to prednisone	20	unknown
Study to evaluate safety and efficacy of AC607 for the treatment of AKI in cardiac surgery subjects	01602328	AlloCure, USA	Allogeneic MSC	200	recruiting
Investigation on autologous MSC in diabetic nephropathy type I	n.a.	Tehran Medical University, Iran	Autologous bmMSCs	20	ongoing
Safety and Efficacy of Mesenchymal Precursor Cells in Diabetic Nephropathy	01843387	Mesoblast, Australia	Allogeneic MPCs 1 dose of 150 mill. or 300 mill.	30	recruiting

Unfractionated adipose tissue has already been used as a stem cell source in clinical trials by the company Cytori®, claiming to have a device which can isolate adipose-derived regenerative cells. The cells isolated with this device have been studied in several clinical trials for cardiovascular diseases including the APOLLO trial where the cell mixture was injected after myocardial infarction (MI). In this situation cell infusion gave a 4% increase in left ventricular function compared to control. It is important to realize that these are not (expanded) ASCs, but a mixture that includes adipose stromal cells, endothelial progenitor cells, leucocytes, endothelial cells and vascular smooth muscle cells and therefore most likely adipose MSCs will be part of this mixture [60]. However, this undefined mixture is very different from characterized adipose tissue derived MSC expansions and therefore the results from these clinical studies are difficult to relate to ongoing clinical studies using bmMSC or cultured ASCs.

Another source of MSCs are MSCs derived from the umbilical cord, either from the blood or from the stroma (Warton Jelly, hWJSCs). hWJSCs can be easily harvested from the umbilical cord, have a higher frequency of proliferation and higher colony-forming unit capacity compared to bmMSCs while having the same immunomodulatory potential. Therefore, as isolation of hWJSCs is non-invasive as opposed to the bone marrow aspiration to acquire bmMSCs and have a higher gain in cell numbers, hWJSCs can easily be banked and may in the end be a very attractive source of MSCs for clinical purposes [61].

3] Autologous or allogeneic MSCs

Until now most studies have used autologous cells. However, due to the expansion period, quality controls and logistics, it takes several weeks to months to manufacture autologous cells, which is a long time for patients in need for treatment. Allogeneic MSCs offer the advantage of availability for clinical use without the delay required for expansion. This is of major importance in the case of indications where the treatment is needed without delay, for example in calcineurin toxicity and allograft rejection. In these indications autologous therapy would only be possible when the cells are harvested in advance, however this is very costly and limits this approach more or less to patient designated therapy. Another theoretical benefit of using allogeneic MSCs is that the age of the donor is controlled, and cells can be selectively derived from young donors. This is important because MSC number and functionality have been shown to decrease with age. Indeed, MSCs derived from older donors showed longer population doubling times, less proliferation and a decrease capacity for osteoblast differentiation [62].

Because of these disadvantages of autologous MSCs, several (even large multi-centre phase III) studies switch to "off the shelf" allogeneic MSCs in diseases as GvHD, autoimmune diseases and vascular diseases [63]. The basis of this switch to use allogenic MSCs for clinical application is the assumption that MSCs do not express HLA class II molecules and costimulatory molecules and have such immunosuppressive properties that they can avoid immune responses entirely, thereby avoiding rejection of the cells. Whether this assumption is based on enough data is extensively reviewed elsewhere [64]. While multiple patients have received allogeneic MSCs in several clinical trials without adverse events related to anti donor immune response, suggesting that no acute immune mediated complications occur, it should be noticed that these were not immune compromised patients such as transplant recipients. At the same time preclinical literature is suggesting that allogeneic MSCs, because they do express HLA class I molecules, can give rise to donor specific T cell and antibody responses. In transplantation there are conflicting preclinical results regarding allogeneic MSCs. Some preclinical studies showed an accelerated graft rejection after allogeneic MSC administration [65,66] while others showed that allogeneic MSCs can work synergistically with immunosuppressive drugs and promote graft survival [16,34,40]. Within the transplantation setting only one clinical study investigated allogeneic, donor derived MSCs [50], however, unfortunately the authors did not look into whether HLA specific antibodies were produced.

In transplantation, the use of specific criteria when applying allogeneic MSCs may minimize the risk of sensitization, these include no sharing of HLA type between MSCs and the kidney donor, and absence of antibodies of the recipient to the MSCs. In addition, immune responses and incidence of allograft rejection, which could be elicited by allogeneic MSCs should be accurately monitored during the course of the study [64]. These assays should include the development of donor specific HLA antibodies (DSA). Alloantibody assays of serum represent a relative simple and highly sensitive means to examine immunogenicity of allogeneic cells and are used routinely in clinical transplant practice to avoid potentially catastrophic antibody-mediated rejection. The importance of these de novo HLA specific DSA as a major cause of allograft failure in the long term has recently been confirmed in numerous studies [67,68]. In most studies, the incidence of these de novo DSA is below 15% [68]. Recently, it was shown that DSA with the ability to activate complement, as determined by this novel C1q assay, are associated with greater risk of acute rejection and allograft loss [69]. Indeed, assessment of the complement-binding capacity of donor-specific anti-HLA antibodies appears to be useful in identifying patients at high risk for kidney allograft loss.

4] MSCs derived from patients with renal disease

As most trials use autologous MSCs, differences in donors can give rise to variations in MSC behavior.

As the first studies with MSCs have been performed with autologous MSCs the question whether kidney function influences the quality and the potency of the MSCs had to be addressed.

We showed that bmMSCs derived from ESRD patients have similar growth potential, characteristics and immunomodulatory potential compared to bmMSCs from healthy controls [70]. This was also reported for human ASCs from patients with renal disease [71]. Others however, showed that exposure to uremic serum gave functional incompetence of murine MSCs [72] and an osteoblast like phenotype in human bmMSCs [73]. Both studies, however, exposed MSCs from healthy donors to uremic conditions and not MSCs derived from renal disease patients. In the latter, at least for ASCs, no effect of exposure to uremic serum was seen [71].

5] Timing, dosage, route and frequency of administration of MSC infusion

Besides differences in MSC isolation and expansion techniques, there is also variation in timing, dosage, route and frequency of administration between different trials. Which factors are important still largely remains to be elucidated.

That these factors can be important is illustrated by looking at the timing of MSC infusion. In a mouse model of graft versus host disease (GvHD), MSCs were most effective when administered after the onset of disease and had no protective effect when administered at the day of bone marrow transplantation, suggesting a pro-inflammatory environment is necessary for MSCs to polarize into an anti-inflammatory phenotype [17]. In contrast, in kidney transplantation, both the preclinical studies as the two clinical trials of Casaraghi and Perico et al. showed a decrease in kidney function when MSCs were administered within a week post transplantation (therefore still in a pro-inflammatory environment after surgery) while this was not seen when MSCs were infused one day prior to transplantation [33,45,46].

Next to the timing also the cell dose should be addressed. In GvHD patients, doses of $0.4x10^6$ to $9\ x10^6$BM MSC per kg body weight have been tested [74]. Doses from $0.8\ x\ 10^6$ were effective, but no clear dose-dependent effect was obtained. In patients that underwent myeloablation, infusion of $1x10^6$ and $2.2x10^6$ MSC per kg body weight showed no toxic effects [75]. Trials in Crohn's disease patients are currently testing doses of $2\ x\ 10^6$ and $8\ x\ 10^6$ bmMSCs per kg body weight, or amounts of $600x10^6$ and $1200x10^6$ MSC per patient [76]. In the POSEIDON trial the

effects of both autologous and allogeneic MSCs were tested in ischemic cardiomyopathy, three different doses were evaluated (resp. 20, 100 and 200x10^6 cells). Interestingly, although all doses improved cardiac function and quality of life, the lowest dose of 20 x10^6 was more effective than the highest dose of 200 x10^6 [77]. Of note, as this differs from previous swine studies where the higher dose was more potent, this emphasizes the differences between animal models and humans and the drawback of translating animal data into clinical trials[78].

Doses of MSC in renal transplantation so far have chosen above what is considered the minimal effective dose, and below a potential toxic dose. Doses of 0.7-2 million cells/kg per infusion were feasible and suggestive for immunosuppression [49]. In particular within the transplantation setting dose finding is of importance as, comparable to other immunosuppressant therapies, a balance between immune suppression to avoid rejection and over immune suppression resulting in opportunistic infections and a higher risk of malignancies has to be found.

Most trials in kidney transplantation up till now have used intravenous administration of cells. In the study of Peng et al. they investigated the direct administration of MSCs within the renal artery, but as the group size is small and donor derived MSCs are delivered as opposed to the autologous MSCs in the other trials, it is not possible to draw conclusions about which route of delivery is more potent [50]. Next to injection of MSCs into the vasculature, MSCs could also be directly injected into the kidney or underneath the kidney capsula, in line with the intramyocardial and transendocardial delivery for cardiac diseases. However, even in cardiac diseases it still remains to be elucidated which method is favorable as there are only a few studies comparing different routes of administration and the results are conflicting [79].

In addition, the frequency of MSC injections is something which should be addressed. Interestingly, in children with steroid resistant graft versus host disease a long lasting response was hardly observed in patients who received one infusion, while most responders had 2 or more infusions [80]. Whether multiple infusions are also necessary in the transplantation setting still needs to be further investigated. The studies currently published all use one to two infusions and the time in between these infusions also varies between studies (fig 2). However, as group sizes are small and protocols differ, it is also difficult to draw conclusions about the frequency of MSC therapy.

6] Potential risks

The potent progenitor as well as the immunomodulatory characteristics of MSCs carry inherent safety risks, including too much immune suppression, tumorigenicity and ectopic tissue formation as reviewed elsewhere [53].

As MSCs are particularly known for their immune suppressive functions a logic side effect of MSC therapy would be over immune suppression. This could also be seen in our phase I trial, where 3 out of 6 patients developed opportunistic virus infections after MSC infusions that are typically associated with too much immune suppression. These included BK nephropathy, primary CMV infection more than 6 months after valganciclovir prophylaxis had been discontinued, and one concerned a CMV reactivation. Of note, in none of the patients that received MSCs regular immune suppression was lowered when the MSCs were given, as they all had signs of rejection or IFTA in the biopsy [49]. In contrast, in the trial of Tan et al. the investigators observed a reduction of opportunistic infections after MSCs therapy. However, CMV donor recipient status was negative in 151 of 154 patients, probably explaining the low incidence of CMV infections in their population [47].

As MSCs can also exhibit a pro-inflammatory phenotype it is of importance to monitor the safety and immune function of MSCs prior to transplantation. Therefore the ISCT-MSC committee has recently proposed standardized methodology and immune assays for MSC isolation and verification of immune suppressive function prior to transplantation with the aim to achieve safe, comparable and unambiguous results on MSC efficacy in clinical trials[81]. Allogeneic MSCs have the highest risk of immunogenicity [64] and anti-donor responses should be monitored accurately as reviewed above.

There has been initial concern with respect to the risk of malignant transformation during the expansion period. More recently, genetic features of MSCs expanded with fetal calf serum (FCS) or with platelet lysates (PL) were tested in 4 cell-therapy facilities during 2 multicenter clinical trials. In this study, some transient and donor-dependent recurring aneuploidy was detected in vitro, independently of the culture process. However, MSCs with or without chromosomal alterations showed progressive growth arrest and entered senescence without evidence of transformation either in vitro or during a 8 week follow up after infusion in immune compromised mice [82]. There is also the risk that genetically normal MSCs may promote the growth of pre-existing tumors. MSCs can be targeted to the tumor by soluble factors such as

stromal cell-derived factor 1 (SDF-1), platelet derived growth factor a (PDGF-a) and VEGF and may be conditioned into tumor-resident MSCs, which can promote tumor growth via e.g. CCL2, thereby recruiting immunosuppressive macrophages[17].

The immune suppressive properties may also decrease immune surveillance of tumors. This is of particular importance in transplant recipients, who, due to the use of immune suppressive drugs already have an increased risk in developing malignancies. Therefore it will be hard to link the development of carcinomas to the use of MSC therapy in transplant recipients. It is clear that long term follow up should be performed in MSC treated patients and one should discuss the extent of screening protocols which should be applied before MSC infusions.

Early study design: small cohorts of patients or large clinical studies

Uncertainties regarding the above mentioned issues raise the question what study design is most suitable in this first phase of clinical trials with MSC therapy and cell therapy in general. Randomized controlled trials (RCT), although they are considered the most powerful trial set up, are not designed to pick up signals that infringe patient safety and require large amounts of patients, making them less suitable for addressing safety considerations and optimizing treatments in the first stages of clinical trials. Another disadvantage of RCTs is the high risks of failure in relationship to the costs, particularly in the setting of cell therapy. One therefore has to consider study designs that harbor adaptive features i.e., changes in design or analyses guided by examination of the accumulated data at interim points so that studies can be more efficient, prevent unnecessary exposure to risk and are more informative (e.g., by providing broader dose-response information)(figure 3). In addition, one could consider establishing registries to follow up on the long term outcome of patients that have been included in MSC studies. Clearly, adaptations during the development program may alter trial conduct and consequently result in a biased assessment of the treatment effect. An Independent Data Monitoring Committee (IDMC) that has well described roles and responsibilities, and that is qualified in methodology therefore needs to be established for an objective and unbiased assessment of the treatment effect of the cell product under investigation.

A particular aspect of the clinical development of MSC therapy is that they are considered an Advanced Therapeutic Medicinal Product (ATMP). This implies that MSCs can only be administered in either one of 3 possible ways: within the setting of a research protocol, as a hospital exemption (for a predefined small number of patients) or as a registered product [83]. As MSCs are by definition a heterogeneous cell mixture that lack unique surface markers, this

implies that the actual fabrication process and not the cell type needs to qualify for a registered status. This would require either the development of randomized clinical trials for each production site, or thorough harmonization of production processes between clinical sites.

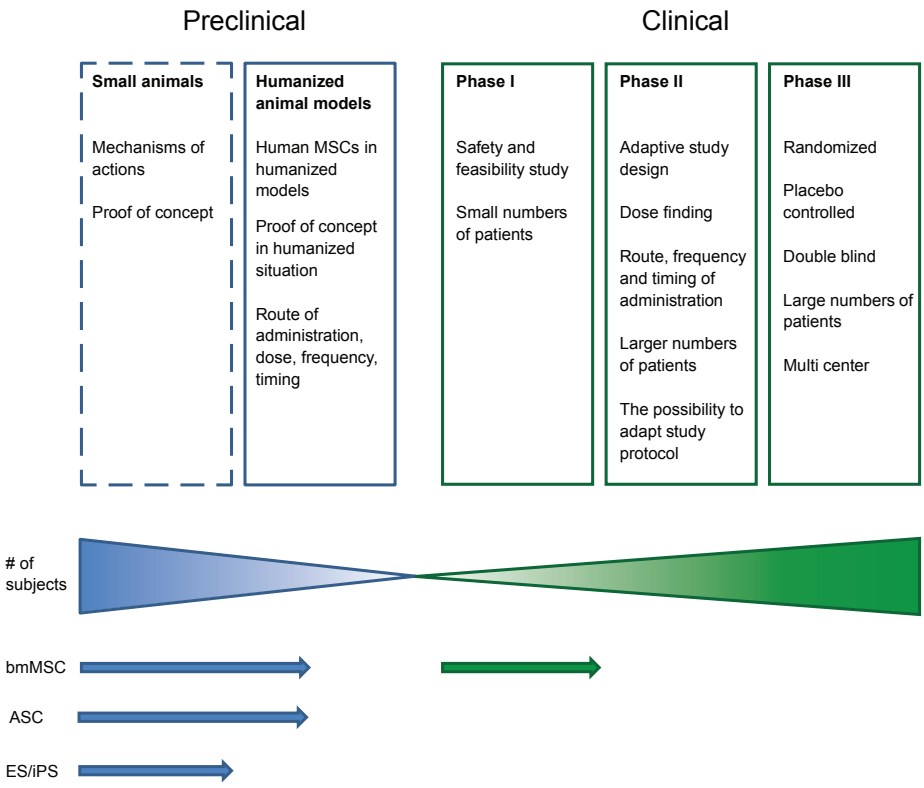


Figure 3. The pathway of cell therapy from preclinical research to clinical translation. The value of small animal models for the human situation is limited and therefore preclinical research should involve the use of human cells in humanized models (such as in transplantation setting a humanized skin graft model) for a proof of concept in a humanized situation. Clinical studies should start with small safety and feasibility studies and first continue with an adaptive study design in order to be able to address dose finding, delivery method and route and frequency of administration before starting large phase III trials.

Future perspectives of cell therapy in kidney transplantation

The focus of cell therapy for kidney transplantation has so far mainly been on MSC therapy. This, however is something which will probably change within the next couple of years as embryonic stem cells (ES) and in particular induced pluripotent stem cells (iPS) are interesting new sources for cell therapy. The NIH center for regenerative medicine recently awarded LONZA Walkersville inc. to generate induced pluripotent stem cells (iPS) under good manufacturing practice (cGMP) [84] . Combining this with the recent exciting results showing that iPS cells can be directed to differentiate into ureteric bud commited progenitor cells [85] and that embryonic stem cells can be directed to both ureteric bud and metanephric mesenchyme, the two main compartments in nephrogenesis [86], gives a new perspective on stem cell therapy in kidney diseases. Either patient derived iPS or iPS derived from an HLA matched databank can be programmed towards kidney progenitor cells and administered as cellular therapy. Whether these cells are safe and potent for tissue repair of course still needs to be addressed, however this new field looks promising.

Another new interesting field is the use of genetically engineered MSCs as a vehicle to deliver cytokines directly into the microenvironment. Here MSCs are transformed with therapeutic genes. For example in Huntington disease this technique is described to deliver brain derived neurotrophic factor and in a rat model of myocardial infarction a novel transfection method of MSCs to overexpress hypoxia inducible VEGF is described [87,88]. It would be interesting to see whether genetically engineered MSCs overexpressing for example anti-inflammatory and anti-fibrotic cytokines and growth factors will enhance kidney repair more potent compared to unmodified MSCs.

Concluding remarks

As MSCs can be both anti-inflammatory and anti-fibrotic, they are of particular interest as a new therapy in kidney disease and organ transplantation. Indeed, in several preclinical models of kidney disease and transplantation, MSC therapy showed a beneficial effect on renal function and graft survival. In some models even a state of tolerance was induced. The first clinical studies with MSC therapy in kidney transplantation showed that MSC therapy is safe and feasible and the first results are promising. However, there are still a lot of questions to be addressed regarding optimal timing, dosage, timing and frequency of infusions. In particular because preclinical data suggest that when MSCs are injected in an anti- or suboptimal inflammatory environment they might not only be less effective but may also exhibit a pro-inflammatory phenotype and therefore may even work counteractive. These are important considerations for setting up new

clinical trials and we therefore recommend to use an adaptive study design for clinical trials with (mesenchymal stromal) cell therapy. In this manner, trials will be safer, more efficient and more informative.

References

1. Levey, A.S. & Coresh, J. Chronic kidney disease. *Lancet* **379**, 165-180.

2. Turin, T.C., *et al.* Lifetime risk of ESRD. *J Am Soc Nephrol* **23**, 1569-1578 (2012).

3. Lamb, K.E., Lodhi, S. & Meier-Kriesche, H.U. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* **11**, 450-462 (2011).

4. DiPersio, J.F. Diabetic stem-cell "mobilopathy". *The New England journal of medicine* **365**, 2536-2538 (2011).

5. Pittenger, M.F., *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* **284**, 143-147 (1999).

6. Friedenstein, A.J., *et al.* Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. *Exp Hematol* **2**, 83-92 (1974).

7. Horwitz, E.M., *et al.* Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* **7**, 393-395 (2005).

8. Ahmad, T., *et al.* Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *The EMBO journal* (2014).

9. Crisan, M., *et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**, 301-313 (2008).

10. Pelekanos, R.A., *et al.* Comprehensive transcriptome and immunophenotype analysis of renal and cardiac MSC-like populations supports strong congruence with bone marrow MSC despite maintenance of distinct identities. *Stem Cell Res* **8**, 58-73 (2012).

11. Bruno, S., *et al.* Isolation and characterization of resident mesenchymal stem cells in human glomeruli. *Stem Cells Dev* **18**, 867-880 (2009).

12. Lee, O.K., *et al.* Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* **103**, 1669-1675 (2004).

13. Pierdomenico, L., *et al.* Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation* **80**, 836-842 (2005).

14. Zuk, P.A., *et al.* Human adipose tissue is a source of multipotent stem cells. *Molecular biology of the cell* **13**, 4279-4295 (2002).

15. Salem, H.K. & Thiemermann, C. Mesenchymal stromal cells: current understanding and clinical status. *Stem Cells* **28**, 585-596 (2010).

16. English, K. & Wood, K.J. Mesenchymal stromal cells in transplantation rejection and tolerance. *Cold Spring Harb Perspect Med* **3**, a015560 (2013).

17. Bernardo, M.E. & Fibbe, W.E. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* **13**, 392-402 (2013).

18. Melief, S.M., *et al.* Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells* **31**, 1980-1991 (2013).

19. Ren, G., *et al.* Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* **2**, 141-150 (2008).

20. Asari, S., *et al.* Mesenchymal stem cells suppress B-cell terminal differentiation. *Exp Hematol* **37**, 604-615 (2009).

21. Schena, F., *et al.* Interferon-gamma-dependent inhibition of B cell activation by bone marrow-derived mesenchymal stem cells in a murine model of systemic lupus erythematosus. *Arthritis and rheumatism* **62**, 2776-2786 (2010).

22. Youd, M., *et al.* Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. *Clinical and experimental immunology* **161**, 176-186 (2010).

23. Spaggiari, G.M., *et al.* Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood* **111**, 1327-1333 (2008).

24. Bianco, P., *et al.* The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nat Med* **19**, 35-42 (2013).

25. Lee, S., *et al.* Distinct macrophage phenotypes contribute to kidney injury and repair. *J Am Soc Nephrol* **22**, 317-326 (2011).

26. Zhang, M.Z., *et al.* CSF-1 signaling mediates recovery from acute kidney injury. *The Journal of clinical investigation* **122**, 4519-4532 (2012).

27. Li, W., *et al.* Mesenchymal stem cells: a double-edged sword in regulating immune responses. *Cell death and differentiation* **19**, 1505-1513 (2012).

28. Khairoun, M., *et al.* Renal ischemia-reperfusion induces a dysbalance of angiopoietins, accompanied by proliferation of pericytes and fibrosis. *American journal of physiology. Renal physiology* **305**, F901-910 (2013).

29. Carmeliet, P. & Jain, R.K. Molecular mechanisms and clinical applications of angiogenesis. *Nature* **473**, 298-307 (2011).

30. Carrion, B., Kong, Y.P., Kaigler, D. & Putnam, A.J. Bone marrow-derived mesenchymal stem cells enhance angiogenesis via their alpha6beta1 integrin receptor. *Experimental cell research* **319**, 2964-2976 (2013).

31. Humphreys, B.D., *et al.* Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *The American journal of pathology* **176**, 85-97 (2010).

32. Wang, Y., He, J., Pei, X. & Zhao, W. Systematic review and meta-analysis of mesenchymal stem/stromal cells therapy for impaired renal function in small animal models. *Nephrology* **18**, 201-208 (2013).

33. Casiraghi, F., *et al.* Localization of Mesenchymal Stromal Cells Dictates Their Immune or Proinflammatory Effects in Kidney Transplantation. *Am J Transplant* **12**, 2373-2383 (2012).

34. Ge, W., *et al.* Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation* **90**, 1312-1320 (2010).

35. Togel, F., Zhang, P., Hu, Z. & Westenfelder, C. VEGF is a mediator of the renoprotective effects of multipotent marrow stromal cells in acute kidney injury. *J Cell Mol Med* **13**, 2109-2114 (2009).

36. Imberti, B., *et al.* Insulin-like growth factor-1 sustains stem cell mediated renal repair. *J Am Soc Nephrol* **18**, 2921-2928 (2007).

37. Bonventre, J.V. Microvesicles from mesenchymal stromal cells protect against acute kidney injury. *J Am Soc Nephrol* **20**, 927-928 (2009).

38. Tomasoni, S., *et al.* Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. *Stem Cells Dev* **22**, 772-780 (2013).

39. Bruno, S., *et al.* Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol* **20**, 1053-1067 (2009).

40. Eggenhofer, E., *et al.* Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front Immunol* **3**, 297 (2012).

41. Dominici, M., *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8**, 315-317 (2006).

42. Fibbe, W.E., Dazzi, F. & LeBlanc, K. MSCs: science and trials. *Nat Med* **19**, 812-813 (2013).

43. Suntharalingam, G., *et al.* Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *The New England journal of medicine* **355**, 1018-1028 (2006).

44. Seok, J., *et al.* Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* **110**, 3507-3512 (2013).

45. Perico, N., *et al.* Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. *Clin J Am Soc Nephrol* **6**, 412-422 (2011).

46. Perico, N., *et al.* Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. *Transpl Int* **26**, 867-878 (2013).

47. Tan, J., *et al.* Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA* **307**, 1169-1177 (2012).

48. Ekberg, H., *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *The New England journal of medicine* **357**, 2562-2575 (2007).

49. Reinders, M.E., *et al.* Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. *Stem Cells Transl Med* **2**, 107-111 (2013).

50. Peng, Y., *et al.* Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. *Transplantation* **95**, 161-168 (2013).

51. Ge, W., *et al.* Infusion of mesenchymal stem cells and rapamycin synergize to attenuate alloimmune responses and promote cardiac allograft tolerance. *Am J Transplant* **9**, 1760-1772 (2009).

52. Streitz, M., *et al.* Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. *Transplantation research* **2**, 17 (2013).

53. Reinders, M.E., Leuning, D.G., de Fijter, J.W., Hoogduijn, M.J. & Rabelink, T.J. Mesenchymal Stromal Cell Therapy for Cardio Renal Disorders. *Current pharmaceutical design* (2013).

54. Horn, P., *et al.* Impact of individual platelet lysates on isolation and growth of human mesenchymal stromal cells. *Cytotherapy* **12**, 888-898 (2010).

55. Casiraghi, F., *et al.* Pretransplant infusion of mesenchymal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells. *J Immunol* **181**, 3933-3946 (2008).

56. Bartholomew, A., *et al.* Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* **30**, 42-48 (2002).

57. Zhou, H.P., *et al.* Administration of donor-derived mesenchymal stem cells can prolong the survival of rat cardiac allograft. *Transplantation proceedings* **38**, 3046-3051 (2006).

58. Franquesa, M., *et al.* Mesenchymal stem cell therapy prevents interstitial fibrosis and tubular atrophy in a rat kidney allograft model. *Stem Cells Dev* **21**, 3125-3135 (2012).

59. Roemeling-van Rhijn, K.M., Korevaar SS, Lievers E, Leuning DG, et al. . Human Bone Marrow-and Adipose Tissue-derived Mesenchymal Stromal Cells are Immunosuppressive In vitro and in a

Humanized Allograft Rejection Model. *J Stem Cell Res Ther* **S6:001. doi: 10.4172/2157-7633.S6-001**(2013).

60. Houtgraaf, J.H., *et al.* First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction. *Journal of the American College of Cardiology* **59**, 539-540 (2012).

61. Bongso, A. & Fong, C.Y. The therapeutic potential, challenges and future clinical directions of stem cells from the Wharton's jelly of the human umbilical cord. *Stem cell reviews* **9**, 226-240 (2013).

62. Zhou, S., *et al.* Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. *Aging cell* **7**, 335-343 (2008).

63. Vaes, B., Van't Hof, W., Deans, R. & Pinxteren, J. Application of MultiStem((R)) Allogeneic Cells for Immunomodulatory Therapy: Clinical Progress and Pre-Clinical Challenges in Prophylaxis for Graft Versus Host Disease. *Front Immunol* **3**, 345 (2012).

64. Griffin, M.D., *et al.* Anti-donor immune responses elicited by allogeneic mesenchymal stem cells: what have we learned so far? *Immunology and cell biology* **91**, 40-51 (2013).

65. Seifert, M., Stolk, M., Polenz, D. & Volk, H.D. Detrimental effects of rat mesenchymal stromal cell pre-treatment in a model of acute kidney rejection. *Front Immunol* **3**, 202 (2012).

66. Inoue, S., *et al.* Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. *Transplantation* **81**, 1589-1595 (2006).

67. Everly, M.J., *et al.* Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation* **95**, 410-417 (2013).

68. Loupy, A., Hill, G.S. & Jordan, S.C. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nature reviews. Nephrology* **8**, 348-357 (2012).

69. Freitas, M.C., *et al.* The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. *Transplantation* **95**, 1113-1119 (2013).

70. Reinders ME, R.-v.R.M., Khairoun M, Lievers E, de Vries DK, Schaapherder AF, Wong SW, Zwaginga JJ, Duijs JM, van Zonneveld AJ, Hoogduijn MJ, Fibbe WE, de Fijter JW, van Kooten C, Rabelink TJ, Roelofs H. Bone marrow-derived mesenchymal stromal cells from patients with end-stage renal disease are suitable for autologous therapy. *Cytotherapy* **Jun;15(6):663-72**(2013).

71. Roemeling-van Rhijn, M., *et al.* Mesenchymal stem cells derived from adipose tissue are not affected by renal disease. *Kidney international* **82**, 748-758 (2012).

72. Noh, H., *et al.* Uremia induces functional incompetence of bone marrow-derived stromal cells. *Nephrol Dial Transplant* **27**, 218-225 (2012).

73. Kramann, R., *et al.* Exposure to uremic serum induces a procalcific phenotype in human mesenchymal stem cells. *Arteriosclerosis, thrombosis, and vascular biology* **31**, e45-54 (2011).

74. Le Blanc, K., *et al.* Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* **371**, 1579-1586 (2008).

75. Koc, O.N., *et al.* Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **18**, 307-316 (2000).

76. www.clinicaltrials.gov.

77. Hare, J.M., *et al.* Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* **308**, 2369-2379 (2012).

78. Schuleri, K.H., *et al.* Autologous mesenchymal stem cells produce reverse remodelling in chronic ischaemic cardiomyopathy. *European heart journal* **30**, 2722-2732 (2009).

79. Sanganalmath, S.K. & Bolli, R. Cell therapy for heart failure: a comprehensive overview of experimental and clinical studies, current challenges, and future directions. *Circulation research* **113**, 810-834 (2013).

80. Ball, L.M., *et al.* Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease. *Br J Haematol* **163**, 501-509 (2013).

81. Krampera, M., *et al.* Immunological characterization of multipotent mesenchymal stromal cells--The International Society for Cellular Therapy (ISCT) working proposal. *Cytotherapy* **15**, 1054-1061 (2013).

82. Tarte, K., *et al.* Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. *Blood* **115**, 1549-1553 (2010).

83. www.ema.europe.eu. *http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000295.jsp&mid=WC0b01ac058007f4bb*.

84. www.lonza.com. *http://www.lonza.com/about-lonza/media-center/news/2013/130220-ipsc-e.aspx*.

85. Xia, Y., *et al.* Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. *Nature cell biology* **15**, 1507-1515 (2013).

86. Takasato, M., *et al.* Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nature cell biology* **16**, 118-126 (2014).

87. Olson, S.D., *et al.* Genetically engineered mesenchymal stem cells as a proposed therapeutic for Huntington's disease. *Molecular neurobiology* **45**, 87-98 (2012).

88. Moon, H.H., *et al.* MSC-based VEGF gene therapy in rat myocardial infarction model using facial amphipathic bile acid-conjugated polyethyleneimine. *Biomaterials* **35**, 1744-1754 (2014).