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Review

Mesenchymal stromal cells for tolerance induction in organ transplantation

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ABSTRACT

The primary challenge in organ transplantation continues to be the need to suppress the host immune system long-term to ensure prolonged allograft survival. Long-term non-specific immunosuppression can, however, result in life-threatening complications. Thus, efforts have been pursued to explore novel strategies that would allow minimization of maintenance immunosuppression, eventually leading to transplant tolerance. In this scenario, bone marrow-derived mesenchymal stromal cells (MSC), given their unique immunomodulatory properties to skew the balance between regulatory and memory T cells, have emerged as potential candidates for cell-based therapy to promote immune tolerance. Here, we review our initial clinical experience with bone marrow-derived MSC in living-donor kidney transplant recipients and provide an overview of the available results of other clinical programs with MSC in kidney and liver transplantation, highlighting hurdles and success of this innovative cell-based therapy.

1. Introduction

The streamlining of surgical techniques and the development of effective immunosuppressive regimens have established organ transplantation as a routine practice for treatment of end-stage organ failure. Unfortunately, the need of long-term non-specific immunosuppressive drugs to control alloimmune response and avoid graft rejection, burdens patients with increased risk of infections, malignancies, cardiovascular and metabolic complications [1–4]. Collective efforts have, therefore, been done to identify and explore novel strategies that would allow minimization of maintenance immunosuppression, limiting the risk of serious side effects while retaining effective anti-rejection potential. At least for kidney transplantation, peri-transplant induction therapy with T cell-depleting antibodies to preventively eliminate alloreactive T cells when the host immune system is exposed for the first time to the graft alloantigens [5] has been considered an useful approach, based on findings of successful minimization of maintenance immunosuppression and early steroid withdrawal [6–8] as well as improvement in short-term kidney transplant outcome [9,10].

T cell-depleting antibodies, including the anti-CD52 monoclonal antibody alemtuzumab and the polyclonal antibody rabbit anti-thymocyte globulin (RATG), eliminate peripheral T cells by activating complement cascade and antibody-dependent cell cytotoxicity [11,12], inducing a profound lymphopenia during the peri-transplant period. There is experimental and clinical evidence that Foxp3$^+$ regulatory T cells (Tregs) are resistant to depletion by induction antibody therapy, particularly that mediated by RATG [13,14]. Moreover, since RATG converts CD4$^+$CD25$^-$ effector T cells into Tregs [15] and promotes their expansion during homeostatic-induced lymphopenia [16], it has been suggested that this T cell depleting antibody could also have a pro-tolerogenic potential. However, the compensatory proliferation of residual T cells also promotes preferential expansion of memory T cells [17–20]. Indeed, compared to their naïve counterpart, memory T cells are more resistant to depletion [21,22] and depletion-resistant memory T cells, especially memory CD8$^+$ T cells [23] undergo a rapid proliferation [24]. Memory T cells represent about half of the total T cell pool and a consistent frequency of them harbors a T cell receptor (TCR) that can cross-react with donor Major-Histocompatibility-Complex (MHC) molecules in a process known as “heterologous immunity” [25–27]. Therefore, the outcome of induction therapies with T cell depleting antibodies is the enrichment of the recipient T cell pool of memory T cells with high potential to predispose to graft rejection, overcoming the possible pro-tolerogenic effects of expanded Tregs. Of note, memory T cells are less susceptible to inhibition by conventional

Abbreviations: BM, bone marrow; CNI, calcineurin inhibitors; CsA, cyclosporine A; MMF, mycophenolate mofetil; MSC, mesenchymal stromal cells; Tregs, regulatory CD4+ T cells; RATG, rabbit anti-thymocyte globulin

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maintenance immunosuppressive drugs with exception of calcineurin inhibitors (CNI) when used at least in vitro at high dose [17,28].

Therefore, the peri-transplant lymphopenic environment induced by T cell depleting antibodies could represent one-shot occasion to reshape the host immune system fostering immunoregulation and eventually a pro-tolerogenic milieu, should expansion of memory T cells during the homeostatic proliferation be prevented. In this scenario, we first thought that a cell therapy approach with Mesenchymal Stromal Cells (MSC), given their unique immunomodulatory properties, could have been a useful strategy to promote immune tolerance to solid organ transplantation in the setting of peri-transplant T cell-depleting induction therapy.

In this review, we provide an overview of the results of our initial pilot clinical studies with bone marrow (BM)-derived MSC in kidney transplant recipients, and summarize the available global experience with MSC in kidney as well as liver transplantation, highlighting hurdles and successes of this innovative cell-based therapy in these settings.

2. Mesenchymal stromal cells and their immunomodulatory profile

Mesenchymal Stromal Cells (MSC) are a heterogeneous population of non-hematopoietic multipotent cells characterized by their ability to differentiate into tissues of mesodermal lineages, such as adipocytes, chondrocytes and osteocytes [29,30]. In 2004 the first clinical evidence of the potent immunosuppressive effects of in vitro culture-expanded MSC isolated from human bone marrow (BM) was provided in a patient with hematologic malignancy [31]. Since then BM was considered the main source of MSC for clinical application, and therefore the most investigated cell population for therapeutic purpose. However, over the last few years, MSC were shown to be present in essentially all adult murine organs and tissues [32] so that several other more accessible MSC sources than BM have been proposed for clinical use, such as adipose tissue [33], dental tissues [34], amniotic fluid [35], placenta [36], Wharton's jelly [37], umbilical cord tissue [38] and cord blood [39].

Due to a lack of unique markers, ten years ago The International Society for Cellular Therapy defined MSC using minimal criteria, which are still the reference for the characterization of these cells at the end of the in vitro isolation and expansion process, namely: plastic adherence under standard culture conditions, expression of CD105, CD73 and CD90 molecules, negative for CD45, CD34, CD19 and CD79 markers, and with the capacity to differentiate into chondrocytes, osteoblasts and adipocytes in vitro [29]. Ex-vivo expanded MSC possess unique immunomodulatory properties. They can affect the effector functions of almost all the cells of the innate [40] and adaptive immune [41–44] systems, both in vitro [45,46] and in vivo [47–50], and both by direct contact with target cells [51–53] and by releasing dozens of soluble molecules [45,50,54]. MSC suppress the proliferation of T cells in response to mitogens [46] or to auto [55] and alloantigens [44,48] in a non-MHC restricted manner. In the perspective of organ transplantation, the most interesting immunological characteristic of MSC is their exquisite capacity of skewing the balance between effector/memory T cells and Foxp3$^+$ regulatory CD4$^+$ T cells (Tregs) [56], driving the alloimmune response toward regulation [41,56]. MSC polarize in vitro both naïve and memory T cells toward Foxp3$^+$ Treg phenotype [53,57–59], a process occurring either during direct co-culture of MSC with purified T cells [53,58,59] or by the generation of intermediate tolerogenic antigen-presenting cells, including dendritic cells [60] or M2 macrophages [61,62]. Several experimental models of MSC-based cell therapy in solid organ transplantation confirmed the ability of MSC to induce long-term graft acceptance through in vivo generation of Tregs [49,56,63–66].

MSC-induced immunosuppression also efficiently targets memory T cells. In-vitro, MSC suppressed the proliferation of human CD45RO$^+$ or CD8$^+$CD28$^-$ memory T cells in response to alloantigens [67]. Of more relevance in the context of T-cell depleting induction therapies, MSC significantly reduced the in vitro proliferation of human CD4$^+$ and CD8$^+$ effector T cells in response to the homeostatic cytokines [55,68], without affecting lymphopenia-induced Treg proliferation [55]. Moreover, in vivo in mice, MSC suppressed in a dose-dependent manner the proliferation and cytotoxic function of memory T cells against alloantigens of both minor [69] and major histocompatibility complexes [67,70].

3. Approaches to the first clinical protocol with MSC in organ transplantation

On the basis of the evidence that MSC favor Treg expansion while constraining memory T cells from homeostatic proliferation, and the encouraging data from experimental models of solid organ transplantation [41,56], we designed a phase I clinical study of BM-derived autologous MSC-based therapy in kidney transplant recipients aimed to assess safety and feasibility of this innovative approach and to explore the impact of this treatment on the host immune response to the allograft. This mainly implied to deal in advance with the choice of the source of BM-derived MSC (autologous or allogeneic), the possible negative impact on MSC functions of biologics and immunosuppressive drugs currently used in transplant recipients, and the selection of appropriate timing of cell infusion, to assure robust experimental conditions that would allow sound responses to our study questions.

3.1. Autologous or allogeneic MSC

Pre-clinical studies in experimental models of organ transplantation documented a comparable ability of autologous and allogeneic MSC to induce Treg expansion and to prolong graft survival [49,71,72], suggesting that autologous MSC are as effective as allogeneic cells in promoting immune-regulation possibly because their low immunogenicity that would limit host immune reaction against cell alloantigens [73]. Although MSC express low level of MHC-I molecules, are negative for MHC-II and costimulatory molecules and are considered low-immunogenic cells [73], other available studies, however, indicate that allogeneic MSC, in fact, could elicit an immune response. In-vivo experiments using allogeneic MSC injection in rodents showed that these cells induce allospecific CD4$^+$ and CD8$^+$ memory T cells [74,75] and the development of alloantibody formation [76–78], raising concerns about possible recipient sensitization in the clinical setting, eventually limiting patient access to a second kidney transplantation if needed. Thus, we reasoned that the introduction of foreign antigens with these cells should be avoided and first pilot clinical studies should begin with autologous MSC, making safety the first objective. On the other hand, in kidney transplant setting, autologous MSC are derived from patients with end-stage renal disease (ESRD) on dialysis or with pre-dialysis severe renal insufficiency, an environment that could underline intrinsic MSC dysfunction. Nonetheless, in vitro data that MSC from ESRD patients retained similar immunomodulatory function to those from healthy subjects [79] were reassuring.

3.2. Impact of immunosuppressive drugs

In our center an immunosuppression minimization protocol is in place that includes induction therapy with basiliximab and low-dose RATG, and maintenance immunosuppression with low-doses of CsA and MMF [80,81]. Steroids are tapered and withdrawn within 6 days after transplantation, when clinically feasible (Fig. 1). Of note, rapamycin combined with MMF in the maintenance immunosuppression regimen following basiliximab and low-dose RATG induction therapy was not as effective as CsA/MMF since the first four patients exposed to this treatment protocol developed acute graft rejection (Remuzzi G, personal communication). Therefore, rapamycin, despite its putative role

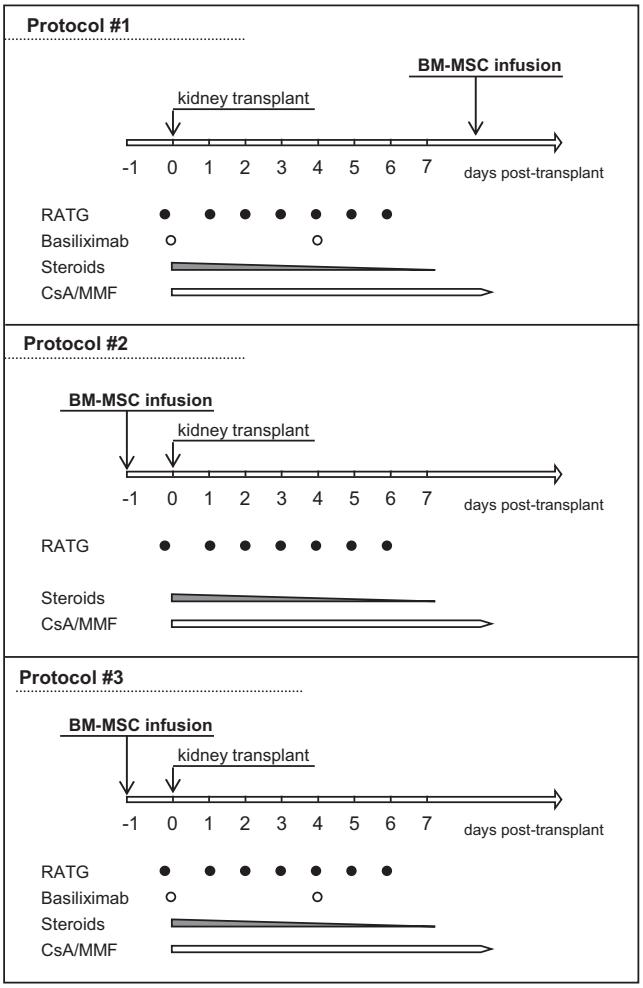

Fig. 1. Clinical protocols of MSC immunotherapy in living-donor kidney transplant recipients. Patients received induction regimen with low-dose RATG infusion (thymoglobulin, 0.5 mg/kg, daily from day 0 to day 6 post-transplant) and basiliximab (20 mg intravenous pre-transplant and on day 4 post-transplant). Five hundred milligrams of methylprednisolone were administered before the first RATG infusion and continued for 2 more days post-transplant (250 and 125 mg, respectively). Subsequently, oral prednisone (75 mg) was administered, which was progressively tapered and discontinued after day 7 post-surgery. Maintenance immunosuppression was with cyclosporine A (CsA) and MMF [83,110]. In protocol #1, two patients received autologous bone marrow-derived (BM)-MSC ($1–2 \times 10^6$/kg body weight) at day 7 after transplantation. In the subsequent protocol #2 BM-MSC infusion was moved before transplantation and basiliximab was removed from the induction therapy (n = 2 patients). A further clinical protocol (protocol #3) foresees pre-transplant (day −1) BM-MSC infusion and induction therapy with both basiliximab and low-dose RATG.

in promoting proliferation/function of Tregs [82] was eventually excluded from the current immunosuppressive protocol adopted in our center.

Thus, we first evaluated whether steroids, CsA and MMF, added in vitro at the same concentration achieved in vivo in kidney allograft recipients, would interfere with MSC immunomodulatory properties [83]. Either steroids or CsA did not negatively affect the capability of MSC to inhibit human T cell proliferation. The ultimate effect of combining MSC with CsA-based immunosuppression remains, however, controversial. Combination of CsA and MSC resulted in impaired immunosuppressive effects in in vitro [84,85] and in vivo mixed lymphocyte reaction (MLR) model [86], whereas CsA synergized with MSC in promoting Treg generation in vitro [87] and in inducing long term graft acceptance of allogeneic islets in rats [88,89], and of vascularized composite transplantation in swine [72,90]. On the other hand, we found that MMF had a positive synergistic effect in vitro with MSC [83]. In line with our finding are in vitro [84,85] and in in-vivo [86] experimental studies showing that the combination of MSC with MMF resulted in a synergistic suppression of $CD4^+$ T cell proliferation in response to allogeneic stimuli. The MSC-MMF synergism has been also documented in heart transplant models in rats [91,92] and in mice [93]. In these settings, MMF synergized with MSC by inhibiting allogeneic MSC-induced T cell activation and trafficking [92] and promoting the conversion of MSC-induced Th-17 cells into Tregs [91].

Examining also the effect of RATG, we found that some polyclonal antibodies within the RATG cocktail dose-dependently bound to MSC in vitro [83]. This finding raised major concerns regarding the possibility of RATG-mediated MSC depletion if the cells are infused during the induction therapy. Indeed, other investigators have shown that RATG, at the doses ranging from 1 to 100 μg/ml, was able to impair human MSC viability and their immunosuppressive functions in vitro. In addition, binding of RATG to MSC increased their susceptibility to be lysed by NKT and cytotoxic $CD8^+$ T cells [94]. On the other hand, we found that the residual amount of RATG present in serum samples collected 7 days after kidney transplantation bound less than 20% MSC without interfering, however, on their capability to inhibit T cell proliferation [83].

These in vitro and ex-vivo studies did guide us on the selection of the most appropriate time for cell infusion in the setting of our T cell depleting induction regimen (Fig. 1).

3.3. Timing of cell infusion

We therefore set MSC infusion at day 7 post-transplantation (Fig. 1), a decision also guided by the fact that this timing corresponded to the start of lymphopenia-induced homeostatic proliferation. This appeared the ideal condition for MSC to promote Treg expansion while exerting their unique ability to curtail memory T cell homeostatic expansion, favoring the development of a pro-tolerogenic milieu.

4. The pilot safety and feasibility study (Clinical protocol #1)

Our first clinical study was with autologous BM-derived MSC infusion in two living related-donor kidney transplant recipients (patient #1 and patient #2) (Fig. 1). MSC were isolated and ex-vivo expanded according to Good-Manufacturing-Practice procedures in the academic "G. Lanzani" Cell-Therapy Laboratory, Bergamo Hospital, Italy (authorization n° aM-189/2008, Agenzia Italiana del Farmaco – AIFA). The treatment protocol was approved by the National Competent Authority and local Ethics Committee [83]. MSC were intravenously infused at day 7 post-transplantation at the dose of 1.7×10^6 cells and 2.0×10^6 cells/kg body weight, respectively. The cell dose was consistent with that previously used for other clinical conditions, all aimed to obtain host immune response modulation [31,95,96], and with the expected number of cells achievable by ex-vivo expansion in a relatively short period of time [97]. MSC infusion was well tolerated with no adverse events and kidney graft function rapidly recovered in the first days post-transplantation [83]. However, both patients developed a mild renal insufficiency 10–14 days after cell infusion with a progressive increase in serum creatinine levels attributed to "engraftment syndrome" according to a graft biopsy that was feasible in patient # 2 [83]. Indeed the kidney biopsy showed an unusual histological picture characterized by a very low number of infiltrating $CD4^+$ and $CD8^+$ T cells B cells and monocytes compared to graft biopsies taken from historical kidney transplant recipients during acute rejection and a high number of infiltrating neutrophils that co-localized with increased deposition of complement C3 [83]. In an attempt to characterize the inflammatory process associated with MSC infusion, biopsy specimens were stained with anti-CD105 and anti-CD44 antibodies markers co-expressed by MSC. A considerable number of $CD105^+CD44^+$ double-positive cells were detected in the biopsy of the MSC-treated patients but not in the control graft biopsies from transplant patients not

receiving the cells, suggesting the recruitment of systemically infused MSC into the kidney allograft [83]. MSC are known to migrate toward the injured kidney [98,99] and evidence is available that MSC can be polarized toward a pro-inflammatory phenotype capable to release pro-inflammatory cytokines and eventually attract neutrophils [100,101]. MSC express several Toll-like receptors (TLR) and a number of endogenous ligands produced upon injury can activate TLR on their surface [102]. According to the type of TLR activation MSC may switch toward a pro-inflammatory or anti-inflammatory phenotype [103]. Ligation of TLR4 converts MSC into a pro-inflammatory phenotype resulting in increased production of IL-8 and macrophage migration inhibitory factor (MIF) that recruit neutrophils and enhance their pro-inflammatory activity [52,104]. Moreover, TLR3 activated-MSC protect neutrophils from apoptosis in IL6/STAT3 IFNβ and GM-CSF dependent manner [105,106]. Thus, in the presence of damage-associated molecular patterns (DAMP) as it occurs during kidney ischemia/reperfusion injury, MSC can promote neutrophil recruitment to the site of injury and protect them from apoptosis. These observations led us to hypothesize that, in the setting of intragraft subclinical inflammatory environment occurring in the first few days post-transplantation, the infused MSC may have been primarily recruited in the graft and activated, eventually amplifying the local inflammatory process to the level affecting graft function (Fig. 2). This hypothesis was tested in a murine kidney transplant model [63], that indeed documented that MSC given few days post-transplant were recruited into the graft, released pro-inflammatory cytokines locally and promoted C3 deposition and intragraft accumulation of neutrophils [63]. These events were avoided by infusing MSC pre-transplantation, a condition that favored MSC localization into the spleen instead of kidney graft, thus recruiting cells in the right place to better promote Treg expansion and a pro-tolerogenic environment [83].

Nevertheless, despite relatively few MSC likely reaching the lymphoid organs in the two patients receiving the infusion post-transplantation, the immunomodulatory and pro-tolerogenic effects of the cell therapy were still documented. Indeed, a marked post-transplant decrease in the percentage of circulating memory $CD8^+$ T cells, the increased ratio between Tregs and memory $CD8^+$ T cells, and the reduction of anti-donor $CD8^+$ T cell cytotoxicity were observed in MSC-treated but not in control kidney transplant recipients given the same immunosuppressive therapy without cells [83]. Both patients who received MSC are healthy with stable graft function after more than 6 year follow-up.

5. The need of revised protocols (Clinical protocol #2 and #3)

Based on the observation of acute renal insufficiency after MSC infusion post-transplantation and the similar findings in mice, the clinical study protocol was implemented moving the cell infusion pre-transplant (Clinical protocol #2) (Fig. 1).

This choice was also supported by the experimental findings that murine ATG induction therapy administered to recipient mice the day of kidney transplantation did not affect the capability of MSC given few hours pre-transplant to induce graft tolerance (unpublished observation). Moreover, there is evidence that after systemic infusion, MSC rapidly disappeared from the circulation (because entrapped in the lungs and due to rapid localization into lymphoid organs [107]), thus being relatively inaccessible to the possible depleting effect of subsequent RATG treatment. Furthermore, we dealt with the additional concern of the possible deleterious effect of the anti-CD25 antibody basiliximab, a component of the induction therapy, on MSC – induced generation of Tregs. Indeed, Tregs express very high levels of CD25 which is relevant for their IL-2-mediated survival and function [108]. Moreover, evidence is available that basiliximab induces a short-term depletion of $CD25^+Foxp3^+$ Tregs in kidney transplant recipients [109], suggesting that this anti-IL2 receptor antibody could neutralize Tregs possibly expanded by MSC given pre-transplantation. With this in mind, in the revised protocol basiliximab was removed from the induction regimen and two additional living-donor kidney transplant recipients enrolled in the new clinical study received induction therapy with low-dose RATG alone and the maintenance immunosuppression with CsA and MMF (Fig. 1) [110].

In both patients (patients #3 and #4) pre-transplant MSC infusion (2×10^6 kg/body weight, i.v.) was uneventful and renal function rapidly improved. In patient #3 renal function remained normal during the current 6 year follow-up, whereas patient #4 experienced biopsy-proven acute cellular rejection 15 days post-transplant, successfully treated with steroid pulses [110]. Renal graft function completely recovered and remained stable thereafter on the long-term. Although in a single MSC-treated patient, this finding was taken to suggest that MSC may have low capacity to control host immune response early post-transplant in the context of highly alloreactive environment that can be achieved in the absence of basiliximab. Indeed, in a matched-cohort

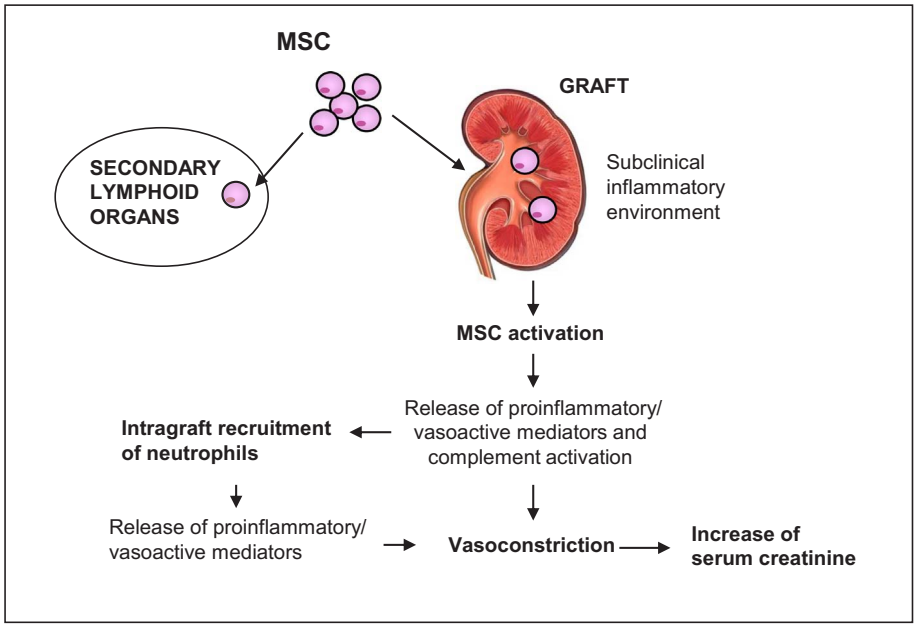

Fig. 2. Proposed mechanism of acute renal dysfunction induced by post-transplant MSC infusion in kidney transplant recipients. The subclinical inflammatory environment of the graft in the few days post-surgery could have favored the prevalent intragraft recruitment of systemically infused BM-MSC. In the graft environment MSC may shift toward a pro-inflammatory phenotype and release neutrophil-chemotactic factors and inflammatory cytokines and contribute to further amplification of complement activation, eventually leading to premature kidney dysfunction (engraftment syndrome).

Table 1
Clinical studies with MSC in solid organ transplantation.

	n patients	MSC source dose and timing	Induction therapy	Maintenance therapy	Treg expansion	Main finding
Kidney transplantation						
Perico (2011) [83]	2	Autologous BM-MSC 1.7–2 × 10^6/kg, iv day + 7	Low-dose RATG + basiliximab	CsA, MMF	Yes	Transient renal insufficiency
Perico (2013) [110]	2	Autologous BM-MSC 2 × 10^6/kg, iv day −1	Low-dose RATG	CsA, MMF	Yes	Acute graft rejection in 1 patient
Mudrabettu (2015) [117]	4	Autologous BM-MSC 0.2–2.8 × 10^6/kg, iv day −1 and day + 30	Low-dose RATG	Tacrolimus, MMF and steroids	Yes	No adverse events, stable graft function during 6 month follow-up
Tan (2012) [118]	156	Autologous BM-MSC 1–2 × 10^6/kg, iv day 0 and day + 14	None-basiliximab only in the control group	CNI standard dose (n = 53 MSC-treated patients, n = 51 controls) or CNI at 80% standard dose (n = 52 MSC-treated patients) plus steroids and MMF	nd	Lower incidence of biopsy-proven acute rejection at 6 but not at 12 months and decreased opportunistic infections in MSC-treated patients compared to controls
Peng (2013) [120] Pan (2016) [119]	16	Donor-derived BM-MSC 5 × 10^6 renal artery, day 0 2 × 10^6/kg, iv, day + 30	Cytoxan	Tacrolimus at 50% standard dose + MMF and steroids	no	MSC combined to low-dose tacrolimus was as effective as standard dose tacrolimus in preventing acute rejection during 2 year follow-up
Reinders (2013) [79]	6	Autologous BM-MSC 0.1–10 × 10^6/kg, iv two infusions seven days apart at month 6–10 post-transplant	Basiliximab	CNI, MMF and steroids	no	Resolution of IF/TA in 2 patients undergoing follow-up biopsy after MSC infusion, increased development of opportunistic infections
Liver transplantation						
Soeder (2015) [112]	1	Allogeneic BM-MAPC 150 × 10^6 intraportal, day 0 150 × 10^6 iv, day + 2	Basiliximab	MMF + tacrolimus from day 6	yes	Clinically diagnosed acute rejection on day 6 and biopsy-proven acute rejection on day 219
Detry (2017) [123]	10	Allogeneic BM-MSC 1.9–2.7 × 10^6/kg, iv 1/3 days post-transplant	None	Tacrolimus, MMF and steroids	no	Similar graft rejection and opportunistic infection episodes as control patients. Failure of immunosuppression withdrawal starting 6 months after transplantation

BM-MSC: Bone Marrow-derived Mesenchymal Stromal Cells; RATG: rabbit anti-thymocyte globulin; CsA: cyclosporine A, MMF: mycophenolate mofetil; IF/TA: interstitial fibrosis/tubular atrophy; CNI: calcineurin inhibitors, MAPC: Multipotent Adult Progenitor Cells, nd: not determined.

observational study we found that induction with low-dose RATG monotherapy was associated with six- to sevenfold higher risk of acute cellular rejection than with the combined regimen of low-dose RATG and basiliximab [111].

As MSC immunomodulatory function develops slowly in the early post-transplant period, adequate induction therapy, including basiliximab, could be of value to limit the risk of acute graft rejection. Nevertheless, these two cases of MSC-treated patients not given basiliximab were also informative to provide evidence that, at least in our setting, the percentage of Tregs in the peripheral blood and their expansion during the first months after transplantation were similar to those in the two initial patients receiving MSC under a combined induction therapy with basiliximab and low-dose RATG [110]. These findings led us to exclude a major negative impact of the anti-CD25 antibody on MSC-induced expansion of peripheral Tregs.

This observation is in keeping with previous demonstration in liver and kidney transplant recipients that basiliximab did not induce significant changes either in the percentage of $Foxp3^+$ or in level of Foxp3 expression in $CD4^+$ T cells [112] or in the inhibitory function of ex-vivo isolated Tregs [113]. There is also evidence that basiliximab down-regulated CD25 expression without compromising the in vitro suppressive function of $CD4^+Foxp3^+$ Tregs [113,114].

Beside the effect on Treg profile we found that in patient #3 and #4 MSC infusion was associated with a reduction of circulating memory $CD8^+$ T cell percentage, particularly remarkable at 6 and 12 months after transplant. This was at variance with findings in control transplant patients given combined induction therapy alone in whom the percentage of memory $CD8^+$ T cells was significantly higher compared to the pre-transplant levels [110]. Consequently, high ratio Treg/memory T cells was observed also in these MSC-treated patients during the early post-transplant period, again anticipating the development of a possible pro-tolerogenic environment supported by the demonstration of donor-specific unresponsiveness of $CD8^+$ T cells in ex-vivo cell-mediated cytotoxicity test [110].

Together, our encouraging findings of the donor-specific immunomodulatory effect of MSC and the major concern of increased risk of acute cellular rejection of induction therapy excluding basiliximab, led us to further modify the clinical protocol which now foresees MSC infusion the day before transplantation and the combined induction regimen with basiliximab and low-dose RATG (Clinical protocol #3, Fig. 1).

6. Long-term outcome of MSC treated patients

Patients treated according to protocol #1 and #2 had stable graft function during the 5–7 year follow-up, without increased susceptibility to infections or malignancy, confirming the safety of MSC therapy even on the long-term in immunosuppressed kidney transplant recipients.

Of interest, extensive immune-monitoring during the long-term follow-up showed a long-lasting sustained increased ratio of circulating Treg/memory $CD8^+$ T cells and donor-specific hyporesponsiveness of $CD8^+$ T cells ex-vivo in two out of four MSC-treated compared to historical kidney transplant recipients given the same induction and maintenance immunosuppressive drugs. In addition, in these two patients we observed a progressive expansion of B cells with a naïve and transitional phenotype, a B cell signature recently reported to be associated with operational tolerance [115,116]. Notably, this pro-tolerogenic environment was particularly evident in a MSC-treated patient, who, therefore, underwent successful withdrawn of CsA, and is currently weaning off the low-dose MMF maintenance immunosuppressive therapy (personal communication).

7. The global experience with MSC in organ transplantation

So far MSC therapy in organ transplantation has been tested mainly in pilot feasibility studies in the setting of kidney and liver transplant programs with different ultimate purposes, namely: i) induction of immune tolerance; ii) replacement of basiliximab induction therapy or minimization of maintenance immunosuppressive drugs; iii) treatment of subclinical rejection and repair of graft tissue injury.

7.1. Kidney transplantation

Similar to our approach, Mudrabettu et al. in India are exploring the pro-tolerogenic properties of autologous BM-derived MSC [117] (Table 1). Four living-donor kidney transplant recipients have been enrolled who were given intravenously two doses of MSC, one day prior to and 30 days post-transplantation (range 0.2 to $2.8x10^6$/kg body weigh/infusion). They also received induction therapy with low-dose RATG and maintenance immunosuppression with tacrolimus, MMF and steroids. All patients had excellent graft function and showed normal graft histology on 1 and 3 month-protocol biopsies. A trend toward increased circulating Tregs was observed during the 3 month follow-up in MSC treated patients compared to control kidney transplant recipients, associated with decreased ex-vivo proliferation of $CD4^+$ T cells [117]. This findings are in line with our results, although the short-term follow-up and the wide range of MSC doses administered in a very small cohort of patients may limit sound conclusions.

Other investigators in China [118] have attempted to reduce the immunosuppressive drug regimens with autologous BM-MSC treatment in a living-donor kidney transplant program. Actually, this is the largest randomized clinical trial in which MSC were given on day 0 and on day 14 post-transplantation ($1–2 \times 10^6$/kg body weight, each i.v. infusion) combined to standard (n = 53 patients) or 80% (n = 52 patients) calcineurin inhibitor (CNI) dose. A control group of patients given basiliximab induction therapy and maintenance immunosuppression with standard CNI dose (n = 52 patients) was considered for comparison. All MSC- and control patients received also steroids and MMF as a part of maintenance immunosuppression. MSC-treated patients showed faster renal function recovery during the first month post-transplant, reduced incidence of biopsy-proven acute rejection at 6 months, not confirmed at 12 months, and a significantly decreased risk of opportunistic infection during the 1 year follow-up, compared to control group [118] (Table 1). Unfortunately, no mechanistic data about the possible immunomodulation afforded by MSC treatment were provided, leaving unexplained the lack of sustained anti-rejection effect of this cell therapy in the setting of minimization of immunosuppressive regimen.

Similarly, another clinical trial in China [119,120] employed allogeneic BM-MSC derived from living kidney donor in 16 transplant recipients as a strategy to reduce maintenance dose of tacrolimus. MSC were administered twice (5×10^6 into the renal artery the day of transplant and 2×10^6/kg body weight, i.v. one month after transplant) and combined to maintenance immunosuppression with MMF, steroids and 50% tacrolimus dose (0.04–0.05 mg/kg/day). Control patients (n = 16) received the same maintenance immunosuppression but with standard tacrolimus dose (0.07–0.08 mg/kg/day). In addition, all patients were given induction therapy with Cytoxan (200 mg/day), a regimen that induced a mild and transient depletion of T cells during the first week after transplant [119,120]. During the 24 month follow-up, the incidence of acute rejection, graft survival and graft function were similar in MSC-treated and control patients, suggesting that MSC allow safe use of sparing dose of CNI [119]. Analysis of peripheral blood lymphocyte population in the first 6 patients [120], however indicated that MSC treatment in this setting did not induce significant changes in Treg percentages compared with controls or basal levels (Table 1).

Other investigators took a different approach and studied MSC treatment to repair kidney graft injury [121]. They performed a safety and feasibility study of autologous BM-derived MSC in six living-donor kidney allograft recipients whose 6 month-protocol biopsy showed subclinical rejection and/or interstitial fibrosis/tubular atrophy (IF/TA). Transplant patients, given basiliximab induction therapy and on

CNI, MMF and steroid immunosuppression, received two iv injections of MSC (dose range for each cell infusion: 0.1–1.1 × 10^6/kg body weight) 7 days apart and followed-up for 24 weeks post-cell infusion. In two recipients who underwent surveillance biopsies after MSC infusion, resolution of tubulitis without IF/TA was documented. Notably, three patients developed opportunistic viral infections, raising concern about possible MSC − induced over-immunosuppression. Proliferation of peripheral blood mononuclear cells in mixed-lymphocyte reactions in response to donor cells was reduced in five out of the six MSC-treated patients after cell infusion. However, no changes in percentages of memory $CD4^+$ and $CD8^+$ T cells or of Tregs was observed after the treatment, indicating mechanisms other than immunoregulation underlying the MSC effect in these patients [121] (Table 1).

7.2. Liver transplantation

First reports about MSC therapy in liver transplantation have been recently made available [122,123] (Table 1). A patient with a living-donor liver transplant was given Multipotent Adult Progenitor Cells (MAPC) [122], a commercial cell product (Multistem, Athersys) isolated and extensively expanded from a single BM aspirate. These cells share similar immunosuppressive function with MSC both in vitro and in vivo settings [122]. MAPC were infused into the portal vein (150 × 10^6 total cells) after allograft reperfusion. Immunosuppressive treatment followed a “bottom-up” CNI-free regimen starting with basiliximab, MMF and steroids (1 mg/kg, tapered off from day 3). A second MAPC dose was administered iv on day 2 post-transplantation (150 × 10^6 total cells). The patient experienced two rejection episodes, the first clinically diagnosed on day 6 and the second biopsy-proven on day 219 [122]. A trend toward increased percentages of circulating Tregs was observed few days after transplant (Table 1).

Recently, Detry et al. [123] evaluated feasibility, safety and tolerability of a single infusion of third-party unrelated BM-MSC in patients with liver transplantation from deceased-donors and attempted, for the first time, immunosuppression drug withdrawal after MSC infusion. Ten liver transplant patients under triple standard immunosuppression (CNI, MMF and steroids) were given MSC infusion (1.9–2.7 × 10^6/kg body weight) 2–5 days post-transplant and were prospectively compared with ten control liver transplant recipients not given the cell treatment during 12 month follow-up. No impairment of liver graft function early after cell infusion or increased incidence of opportunistic infections or malignancies were reported. Rate of rejection, graft survival as well as Banff and fibrosis scores on 6 months protocol biopsies were similar between MSC-treated and control patients. A detailed analysis of peripheral blood T cells showed comparable percentages of total Tregs or their naïve, resting, activated and proliferating Treg subsets, clearly excluding any possible effect of MSC on Treg expansion at least during the initial 3 month follow-up (Table 1). Nevertheless, in 9 MSC recipients progressive immunosuppression weaning was attempted from month 6 to 12 post-transplantation. Notably, in a patient tacrolimus first and then MMF withdrawal was successfully performed and the patient remained off immunosuppression for the subsequent 12 month follow-up. In two patients tacrolimus withdrawal was achieved at month 9 post-transplant but was then reintroduced during weaning MMF monotherapy because of acute graft rejection. In six other patients the planned tacrolimus withdrawal was stopped due to significant increase in transaminases during drug tapering, anticipating the ongoing acute rejection [123].

The very fast tapering of tacrolimus during 3 months could account for the failure of immunosuppressive drug withdrawal in this study. Drug discontinuation within this short-term could have promoted effector T cell activation, disrupting any possible MSC-induced immunomodulation. In addition, in these MSC-treated liver transplant recipients there was no evidence of the development of a pro-tolerogenic environment which would have justified the attempt to and safely guided drug tapering in selected patients. The lack of induction therapy with T-cell depleting antibodies and the MSC infusion early post-transplant instead of pre-transplant could have also contributed to hinder the development of a pro-tolerogenic milieu.

8. The way to transplant tolerance with MSC

Knowledge about MSC in organ transplantation is still too limited to embark on large randomized clinical trials aimed at immune tolerance, and many questions remain regarding the mechanisms of action in humans, the most appropriate dose regimen and way of administration, as well as the benefits of this cell therapy [124]. However, the available data in kidney and liver transplantation have clearly documented that infusion of BM-derived MSC, at least at doses ranging 1–2 × 10^6/kg body weight is rather safe, well tolerated, and without significant side effects in the short- and long-term, even in chronically immunosuppressed patients, a key step for future clinical development of this innovative cell therapy in the transplant setting. The preliminary experience also indicates that MSC are capable of dampening alloimmune response allowing to minimize the dosage of current immunosuppressive biologics and drugs, at least in low-risk kidney transplant recipients. These findings, however, raise the question whether a costly MSC-based therapy should be adopted just to prevent acute allograft rejection and maintain acceptable long-term graft function without induction therapy or with minimized maintenance immunosuppression, all transplant outcomes that are well controlled by conventional, less expensive immunosuppressive drugs. Instead, the final goal of MSC therapy in organ transplantation should be furthering donor-specific immune tolerance, allowing discontinuation of all maintenance immunosuppressive drugs after a certain time post-transplantation. Despite data supporting a degree of MSC-induced donor-specific unresponsiveness to allograft, only anecdotal cases have provided evidence that MSC-based therapy enables the almost complete withdrawal of immunosuppressive drugs or even promotes operational tolerance. This could be attributed to the fact that, unfortunately, not all kidney or liver transplant recipients who were given MSC treatment consistently had signs of immunoregulation. A possible explanation could be that MSC preparations display substantial donor-to-donor variability in their capability to dampen the alloimmune response in vitro, despite common immunophenotype and tri-lineage differentiation potential [125]. In addition, other factors such as isolation and expansion methods, primary cell source and donor characteristics impact considerably on the immunosuppressive properties and on the secretome of the MSC final product [126]. To overcome these important limitations, small and medium enterprises are investing in the development of a very-well characterized, pure, safe and effective MSC product starting from more characterized progenitor cells [126] which will eventually translate in an economically affordable MSC cell therapy.

Also, the host microenvironment surrounding MSC, which may influence their polarization toward a tolerogenic or inflammatory phenotype, should play a role in the heterogeneous immunoregulatory response induced by MSC therapy, that could be limited by planning the cell infusion pre-transplantation to avoid “engraftment syndrome”. Moreover, it appears relevant to facilitate the development of a pro-tolerogenic milieu by administering MSC just before a peri-transplant lymphopenic environment is induced by T cell-depleting antibodies, such as RATG. Together, these observations highlight the hazard of testing MSC-based therapy as a strategy for promoting graft tolerance in unselected cohorts of transplant recipients within the framework of scheduled immunosuppression withdrawal protocols just based on clinical criteria. Instead, we would anticipate the need of applying a comprehensive immunomonitoring approach, including T and B cell subset phenotyping and ex-vivo functional assays, to demonstrate the efficacy of MSC in promoting donor-specific immunoregulation and developing a pro-tolerogenic environment in transplant recipients, which would provide sound criteria for identifying candidate patients

amenable to immunosuppressive drug withdrawal at a given time after transplantation.

Although much work has to be done to fully exploit the pro-tolerogenic potential of MSC in the setting of solid organ transplantation, we look forward with optimism to the next decade where we hope the current encouraging and promising preliminary results may eventually pave the way to definitely demonstrate, through well-designed clinical trials including standardized immunomonitoring, effectiveness and suitability of the BM-derived MSC in inducing sustained operational tolerance in transplant patients.

References

[1] C.N. Kotton, J.A. Fishman, Viral infection in the renal transplant recipient, J. Am. Soc. Nephrol. 16 (2005) 1758–1774.
[2] J.R. Chapman, A.C. Webster, G. Wong, Cancer in the transplant recipient, Cold Spring Harb. Perspect. Med. 3 (2013) a015677.
[3] S. Stoumpos, A.G. Jardine, P.B. Mark, Cardiovascular morbidity and mortality after kidney transplantation, Transpl. Int. 25 (2015) 10–21.
[4] N. Tufton, S. Ahmad, C. Rolfe, R. Rajkariar, C. Byrne, T.A. Chowdhury, New-onset diabetes after renal transplantation, Diabet. Med. 31 (2014) 1284–1292.
[5] E. Page, J. Kwun, B. Oh, S. Knechtle, Lymphodepletional strategies in transplantation, Cold Spring Harb. Perspect. Med. 3 (2013) a015511.
[6] E.S. Woodle, M.R. First, J. Pirsch, F. Shihab, A.O. Gaber, P. Van Veldhuisen, A prospective, randomized, double-blind, placebo-controlled multicenter trial comparing early [7 day] corticosteroid cessation versus long-term, low-dose corticosteroid therapy, Ann. Surg. 248 (2008) 564–577.
[7] K.L. Lentine, M.A. Schnitzler, H. Xiao, D.C. Brennan, Long-term safety and efficacy of antithymocyte globulin induction: use of integrated national registry data to achieve ten-year follow-up of 10–10 Study participants, Trials 16 (2015) 365.
[8] R. Margreiter, J. Klempnauer, P. Neuhaus, F. Muehlbacher, C. Boesmueller, R.Y. Calne, Alemtuzumab [Campath-1H] and tacrolimus monotherapy after renal transplantation: results of a prospective randomized trial, Am. J. Transplant. 8 (2008) 1480–1485.
[9] D.C. Brennan, J.A. Daller, K.D. Lake, D. Cibrik, D. Del Castillo, Rabbit antithymocyte globulin versus basiliximab in renal transplantation, N. Engl. J. Med. 355 (2006) 1967–1977.
[10] G. Mourad, E. Morelon, C. Noel, D. Glotz, Y. Lebranchu, The role of Thymoglobulin induction in kidney transplantation: an update, Clin. Transplant. 26 (2012) E450–E464.
[11] A.D. Kirk, D.A. Hale, R.B. Mannon, D.E. Kleiner, S.C. Hoffmann, R.L. Kampen, et al., Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab [CAMPATH-1H], Transplantation 76 (2003) 120–129.
[12] X. Preville, M. Flacher, B. LeMauff, S. Beauchard, P. Davelu, J. Tiollier, et al., Mechanisms involved in antithymocyte globulin immunosuppressive activity in a nonhuman primate model, Transplantation 71 (2001) 460–468.
[13] A. Kroemer, X. Xiao, M.D. Vu, W. Gao, K. Minamimura, M. Chen, M, et al., OX40 controls functionally different T cell subsets and their resistance to depletion therapy, J. Immunol. 179 (2007) 5584–5591.
[14] S. Gurkan, Y. Luan, N. Dhillon, S.R. Allam, T. Montague, J.S. Bromberg, et al., Immune reconstitution following rabbit antithymocyte globulin, Am. J. Transplant. 10 (2010) 2132–2141.
[15] M. Lopez, M.R. Clarkson, M. Albin, M.H. Sayegh, N. Najafian, A novel mechanism of action for anti-thymocyte globulin: induction of CD4 + CD25 + Foxp3 + regulatory T cells, J. Am. Soc. Nephrol. 17 (2006) 2844–2853.
[16] X. Feng, S. Kajigaya, E.E. Solomou, K. Keyvanfar, X. Xu, N. Raghavachari, et al., Rabbit ATG but not horse ATG promotes expansion of functional CD4 + CD25highFOXP3 + regulatory T cells in vitro, Blood 111 (2008) 3675–3683.
[17] J.M. Rosenblum, A.D. Kirk, Recollective homeostasis and the immune consequences of peritransplant depletional induction therapy, Immunol. Rev. 258 (2014) 167–182.
[18] V.F. Moxham, J. Karegli, R.E. Phillips, K.L. Brown, T.T. Tapmeier, R. Hangartner, et al., Homeostatic proliferation of lymphocytes results in augmented memory-like function and accelerated allograft rejection, J. Immunol. 180 (2008) 3910–3918.
[19] K. Ayasoufi, H. Yu, R. Fan, X. Wang, J. Williams, A. Valujskikh, Pretransplant antithymocyte globulin has increased efficacy in controlling donor-reactive memory T cells in mice, Am. J. Transplant. 13 (2013) 589–599.
[20] D.C. Neujahr, C. Chen, X. Huang, J.F. Markmann, S. Cobbold, H. Waldmann, et al., Accelerated memory cell homeostasis during T cell depletion and approaches to overcome it, J. Immunol. 176 (2006) 4632–4639.
[21] J.P. Pearl, J. Parris, D.A. Hale, S.C. Hoffmann, W.B. Bernstein, K.L. McCoy, et al., Immunocompetent T-cells with a memory-like phenotype are the dominant cell type following antibody-mediated T-cell depletion, Am. J. Transplant. 5 (2005) 465–474.
[22] M. Noris, F. Casiraghi, M. Todeschini, P. Cravedi, D. Cugini, G. Monteferrante, et al., Regulatory T cells and T cell depletion: role of immunosuppressive drugs, J. Am. Soc. Nephrol. 18 (2007) 1007–1018.
[23] C.L. Mackall, T.A. Fleisher, M.R. Brown, M.P. Andrich, C.C. Chen, I.M. Feuerstein, et al., Distinctions between CD8 + and CD4 + T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy, Blood 89 (1997) 3700–3707.
[24] N.K. Tchao, L.A. Turka, Lymphodepletion and homeostatic proliferation: implications for transplantation, Am. J. Transplant. 12 (2012) 1079–1090.
[25] R. Lechler, G. Lombardi, Structural aspects of allorecognition, Curr. Opin. Immunol. 3 (1991) 715–721.
[26] B. Pantenburg, F. Heinzel, L. Das, P.S. Heeger, A. Valujskikh, T cells primed by Leishmania major infection cross-react with alloantigens and alter the course of allograft rejection, J. Immunol. 169 (2002) 3686–3693.
[27] A.L. Amir, L.J. D'Orsogna, D.L. Roelen, M.M. van Loenen, R.S. Hagedoorn, R. de Boer, et al., Allo-HLA reactivity of virus-specific memory T cells is common, Blood 115 (2010) 3146–3157.
[28] A.J. Page, M.L. Ford, A.D. Kirk, Memory T-cell-specific therapeutics in organ transplantation, Curr. Opin. Organ Transplant. 14 (2009) 643–649.
[29] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, et al., Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317.
[30] A.J. Friedenstein, S. Piatetzky II, K.V. Petrakova, Osteogenesis in transplants of bone marrow cells, J. Embryol. Exp. Morphol. 16 (1966) 381–390.
[31] K. Le Blanc, I. Rasmusson, B. Sundberg, C. Gotherstrom, M.M. Hassan, M.H. Uzunel, et al., Treatment of severe acute graft-versus-host disease with third-party haploidentical mesenchymal stem cells, Lancet 363 (2004) 1439–1441.
[32] L. da Silva Meirelles, P.C. Chagastelles, N.B. Nardi, Mesenchymal stem cells reside in virtually all post-natal organs and tissues, J. Cell. Sci. 119 (2006) 2204–2213.
[33] H. Orbay, M. Tobita, H. Mizuno, Mesenchymal stem cells isolated from adipose and other tissues: basic biological properties and clinical applications, Stem Cells Int. 2012 (2012) 461718.
[34] M. La Noce, F. Paino, A. Spina, P. Naddeo, R. Montella, V. Desiderio, et al., Dental pulp stem cells: state of the art and suggestions for a true translation of research into therapy, J. Dent. 42 (2014) 761–768.
[35] P.S. In't Anker, S.A. Scherjon, C. Kleijburg-van der Keu, W.A. Noort, F.H. Claas, R. Willemze, et al., Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation, Blood 102 (2003) 1548–1549.
[36] P.S. In't Anker, S.A. Scherjon, C. Kleijburg-van der Keur, G.M. de Groot-Swings, F.H. Claas, W.E. Fibbe, et al., Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta, Stem Cells 22 (2004) 1338–1345.
[37] A.K. Batsali, M.C. Kastrinaki, H.A. Papadaki, C. Pontikoglou, Mesenchymal stem cells derived from Wharton's Jelly of the umbilical cord: biological properties and emerging clinical applications, Curr. Stem. Cell. Res. Ther. 8 (2013) 144–155.
[38] R. Sarugaser, D. Lickorish, D. Baksh, M.M. Hosseini, J.E. Davies, Human umbilical cord perivascular [HUCPV] cells: a source of mesenchymal progenitors, Stem Cells 23 (2005) 220–229.
[39] A. Flynn, F. Barry, T. O'Brien, T, UC blood-derived mesenchymal stromal cells: an overview, Cytotherapy 9 (2007) 717–726.
[40] K. Le Blanc, D. Mougiakakos, Multipotent mesenchymal stromal cells and the innate immune system, Nat. Rev. Immunol. 12 (2012) 383–396.
[41] F. Casiraghi, N. Perico, M. Cortinovis, G. Remuzzi, Mesenchymal stromal cells in renal transplantation: opportunities and challenges, Nat. Rev. Nephrol. 12 (2016) 241–253.
[42] K. English, F.P. Barry, B.P. Mahon, Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presentation, Immunol. Lett. 115 (2008) 50–58.
[43] S. Beyth, Z. Borovsky, D. Mevorach, M. Liebergall, Z. Gazit, H. Aslan, et al., Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness, Blood 105 (2005) 2214–2219.
[44] S. Aggarwal, M.F. Pittenger, Human mesenchymal stem cells modulate allogeneic immune cell responses, Blood 105 (2005) 1815–1822.
[45] G.M. Spaggiari, H. Abdelrazik, F. Becchetti, L. Moretta, MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2, Blood 113 (2009) 6576–6583.
[46] M. Di Nicola, C. Carlo-Stella, M. Magni, M. Milanesi, P.D. Longoni, P. Matteucci, et al., Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli, Blood 99 (2002) 3838–3843.
[47] S. Chiesa, S. Morbelli, S. Morando, M. Massollo, C. Marini, A. Bertoni, et al., Mesenchymal stem cells impair in vivo T-cell priming by dendritic cells, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 17384–17389.
[48] A. Bartholomew, C. Sturgeon, M. Siatskas, K. Ferrer, K. McIntosh, S. Patil, et al., Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo, Exp. Hematol. 30 (2002) 42–48.
[49] F. Casiraghi, N. Azzollini, P. Cassis, B. Imberti, M. Morigi, D.D. Cugini, 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 (2008) 3933–3946.
[50] K. Akiyama, C. Chen, D. Wang, X. Xu, C. Qu, T. Yamaza, et al., Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis, Cell. Stem. Cell. 10 (2012) 544–555.
[51] F. Gieseke, J. Bohringer, R. Bussolari, M. Dominici, R. Handgretinger, I. Muller, Human multipotent mesenchymal stromal cells use galectin-1 to inhibit immune effector cells, Blood 116 (2010) 3770–3779.
[52] R.G. Ren, L. Zhang, X. Zhao, G. Xu, Y. Zhang, A.I. Roberts, et al., Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide, Cell. Stem. Cell. 2 (2008) 141–150.
[53] K. English, J.M. Ryan, L. Tobin, M.J. Murphy, F.P. Barry, B.P. Mahon, Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4 + CD25(High) forkhead

box P3+ regulatory T cells, Clin. Exp. Immunol. 156 (2009) 149–160.
[54] K. Sato, K. Ozaki, I. Oh, A. Meguro, K. Hatanaka, T. Nagai, et al., Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells, Blood 109 (2007) 228–234.
[55] J.L. Reading, J.H. Yang, S. Sabbah, A. Skowera, R.R. Knight, J. Pinxteren, et al., Clinical-grade multipotent adult progenitor cells durably control pathogenic T cell responses in human models of transplantation and autoimmunity, J. Immunol. 1090 (2013) 4542–4552.
[56] F. Casiraghi, N. Perico, G.G. Remuzzi, Mesenchymal stromal cells to promote solid organ transplantation tolerance, Curr. Opin. Organ. Transplant. 18 (2013) 51–58.
[57] M. Di Ianni, B. Del Papa, M. De Ioanni, L. Moretti, E. Bonifacio, D. Cecchini, et al., Mesenchymal cells recruit and regulate T regulatory cells, Exp. Hematol. 36 (2008) 309–318.
[58] R. Maccario, M. Podesta, A. Moretta, A. Cometa, P. Comoli, D. Montagna, et al., Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype, Haematologica 90 (2005) 516–525.
[59] C. Prevosto, M. Zancolli, P. Canevali, M.R. Zocchi, A. Poggi, Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction, Haematologica 92 (2007) 881–888.
[60] Z.G. Zhao, W. Xu, L. Sun, Y. You, F. Li, Q.B. Li, et al., Immunomodulatory function of regulatory dendritic cells induced by mesenchymal stem cells, Immunol. Invest. 41 (2012) 183–198.
[61] J.H. Ylostalo, T.J. Bartosh, K. Coble, D.J. Prockop, Human mesenchymal stem/stromal cells cultured as spheroids are self-activated to produce prostaglandin E2 that directs stimulated macrophages into an anti-inflammatory phenotype, Stem Cells. 30 (2012) 2283–2296.
[62] M. Francois, R. Romieu-Mourez, M. Li, J. Galipeau, Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation, Mol. Ther. 20 (2012) 187–195.
[63] F. Casiraghi, N. Azzollini, M. Todeschini, R.A. Cavinato, P. Cassis, S. Solini, et al., Localization of mesenchymal stromal cells dictates their immune or proinflammatory effects in kidney transplantation, Am. J. Transplant. 12 (2012) 2373–2383.
[64] Y. Ding, D. Xu, G. Feng, A. Bushell, R.J. Muschel, K.J. Wood, Mesenchymal stem cells prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9, Diabetes 58 (2009) 1797–1806.
[65] D.M. Xu, X.F. Yu, D. Zhang, M.X. Zhang, J.F. Zhou, P.H. Tan, et al., Mesenchymal stem cells differentially mediate regulatory T cells and conventional effector T cells to protect fully allogeneic islet grafts in mice, Diabetologia 55 (2012) 1091–1102.
[66] W. Ge, J. Jiang, J. Arp, W. Liu, B. Garcia, H. Wang, Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression, Transplantation 90 (2010) 1312–1320.
[67] A.U. Engela, C.C. Baan, N.H. Litjens, M. Franquesa, M.G. Betjes, W. Weimar, et al., Mesenchymal stem cells control alloreactive CD8(+) CD28(-) T cells, Clin. Exp. Immunol. 174 (2013) 449–458.
[68] J.L. Reading, B. Vaes, C. Hull, S. Sabbah, T. Hayday, N.S. Wang, et al., Suppression of IL-7-dependent effector T-cell expansion by multipotent adult progenitor cells and PGE2, Mol. Ther. 23 (2015) 1783–1793.
[69] M. Krampera, S. Glennie, J. Dyson, D. Scott, R. Laylor, E. Simpson, et al., Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide, Blood 101 (2003) 3722–3729.
[70] H. Karlsson, S. Samarasinghe, L.M. Ball, B. Sundberg, A.C. Lankester, F. Dazzi, et al., Mesenchymal stem cells exert differential effects on alloantigen and virus-specific T-cell responses, Blood 112 (2008) 532–541.
[71] Y. Wang, A. Zhang, Z. Ye, H. Xie, S. Zheng, Bone marrow-derived mesenchymal stem cells inhibit acute rejection of rat liver allografts in association with regulatory T-cell expansion, Transplant. Proc. 41 (2009) 4352–4356.
[72] Y.R. Kuo, C.C. Chen, H.S. Shih, S. Goto, C.W. Huang, C.T. Wang, et al., Prolongation of composite tissue allotransplant survival by treatment with bone marrow mesenchymal stem cells is correlated with T-cell regulation in a swine hind-limb model, Plast. Reconstr. Surg. 127 (2011) 569–579.
[73] J.A. Ankrum, J.F. Ong, J.M. Karp, Mesenchymal stem cells: immune evasive, not immune privileged, Nat. Biotechnol. 32 (2014) 252–260.
[74] L. Zangi, R. Margalit, S. Reich-Zeliger, E. Bachar-Lustig, A. Beilhack, R.R. Negrin, et al., Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells, Stem. Cells. 27 (2009) 2865–2874.
[75] N. Eliopoulos, J. Stagg, L. Lejeune, S. Pommey, J. Galipeau, Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice, Blood 106 (2005) 4057–4065.
[76] A.T. Badillo, K.J. Beggs, E.H. Javazon, J.C. Tebbets, A.W. Flake, Murine bone marrow stromal progenitor cells elicit an in vivo cellular and humoral alloimmune response, Biol. Blood Marrow Transplant. 13 (2007) 412–422.
[77] K.J. Beggs, A. Lyubimov, J.N. Borneman, A. Bartholomew, A. Moseley, R. Dodds, et al., Immunologic consequences of multiple, high-dose administration of allogeneic mesenchymal stem cells to baboons, Cell. Transplant. 15 (2006) 711–721.
[78] A.J. Poncelet, Y. Nizet, J. Vercruysse, A.L. Hiel, A. Saliez, P. Gianello, Inhibition of humoral response to allogeneic porcine mesenchymal stem cell with 12 days of tacrolimus, Transplantation 86 (2008) 1586–1595.
[79] M.E. Reinders, M. Roemeling-van Rhijn, M. Khairoun, E. Lievers, D.K. de Vries, A.F. Schaapherder, et al., Bone marrow-derived mesenchymal stromal cells from patients with end-stage renal disease are suitable for autologous therapy, Cytotherapy 15 (2013) 663–672.
[80] P. Ruggenenti, I. Codreanu, P. Cravedi, A. Perna, E. Gotti, G. Remuzzi, Basiliximab combined with low-dose rabbit anti-human thymocyte globulin: a possible further step toward effective and minimally toxic T cell-targeted therapy in kidney transplantation, Clin. J. Am. Soc. Nephrol. 1 (2006) 546–554.
[81] A. Gennarini, P. Cravedi, M. Marasa, A. Perna, G. Rota, M. Bontempelli, et al., Perioperative minimal induction therapy: a further step toward more effective immunosuppression in transplantation, J. Transplant. 2012 (2012) 426042.
[82] J. Shan, L. Feng, Y. Li, G. Sun, X. Chen, P. Chen, The effects of rapamycin on regulatory T cells: its potential time-dependent role in inducing transplant tolerance, Immunol. Lett. 162 (2014) 74–86.
[83] N. Perico, F. Casiraghi, M. Introna, E. Gotti, M. Todeschini, R.A. Cavinato, et al., Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility, Clin. J. Am. Soc. Nephrol. 6 (2011) 412–422.
[84] M.J. Hoogduijn, M.J. Crop, S.S. Korevaar, A.M. Peeters, M. Eijken, L.P. Maat, et al., Susceptibility of human mesenchymal stem cells to tacrolimus, mycophenolic acid, and rapamycin, Transplantation 86 (2008) 1283–1291.
[85] F. Buron, H. Perrin, C. Malcus, O. Hequet, O. Thaunat, M.N. Kholopp-Sarda, et al., Human mesenchymal stem cells and immunosuppressive drug interactions in allogeneic responses: an in vitro study using human cells, Transplant. Proc. 41 (2009) 3347–3352.
[86] E. Eggenhofer, P. Renner, Y. Soeder, F.C. Popp, M.J. Hoogduijn, E.K. Geissler, et al., Features of synergism between mesenchymal stem cells and immunosuppressive drugs in a murine heart transplantation model, Transpl. Immunol. 25 (2011) 141–147.
[87] M. Hajkova, B. Hermankova, E. Javorkova, P. Bohacova, A. Zajicova, V. Holan, et al., Mesenchymal stem cells attenuate the adverse effects of immunosuppressive drugs on distinct T Cell subopulations, Stem. Cell. Rev. 13 (2017) 104–115.
[88] M.G. Solari, S. Srinivasan, I. Boumaza, J. Unadkat, G. Harb, A. Garcia-Ocana, et al., Marginal mass islet transplantation with autologous mesenchymal stem cells promotes long-term islet allograft survival and sustained normoglycemia, J. Autoimmun. 32 (2009) 116–124.
[89] Y.H. Kim, Y.M. Wee, M.Y. Choi, D.G. Lim, S.C. Kim, D.J. Han, Interleukin [IL]-10 induced by CD11b[+] cells and IL-10-activated regulatory T cells play a role in immune modulation of mesenchymal stem cells in rat islet allografts, Mol. Med. 17 (2011) 697–708.
[90] Y.R. Kuo, C.C. Chen, S. Goto, Y.T. Huang, C.T. Wang, C.C. Tsai, et al., Immunomodulatory effects of bone marrow-derived mesenchymal stem cells in a swine hemi-facial allotransplantation model, PLoS One 7 (2012) e35459.
[91] F.C. Popp, E. Eggenhofer, P. Renner, P. Slowik, S.A. Lang, H. Kaspar, et al., Mesenchymal stem cells can induce long-term acceptance of solid organ allografts in synergy with low-dose mycophenolate, Transplant. Immunol. 20 (2008) 55–60.
[92] E. Eggenhofer, J.F. Steinmann, P. Renner, P. Slowik, P. Piso, E.K. Geissler, et al., Mesenchymal stem cells together with mycophenolate mofetil inhibit antigen presenting cell and T cell infiltration into allogeneic heart grafts, Transpl. Immunol. 24 (2011) 157–163.
[93] N. Obermajer, F.C. Popp, Y. Soeder, J. Haarer, E.K. Geissler, H.J. Schlitt, et al., Conversion of Th17 into IL-17A[neg] regulatory T cells: a novel mechanism in prolonged allograft survival promoted by mesenchymal stem cell-supported minimized immunosuppressive therapy, J. Immunol. 190 (2014) 4988–4999.
[94] M. Franquesa, C.C. Baan, S.S. Korevaar, A.U. Engela, M. Roemeling-van Rhijn, W. Weimar, et al., The effect of rabbit antithymocyte globulin on human mesenchymal stem cells, Transpl. Int. 26 (2013) 651–658.
[95] D. Karussis, C. Karageorgiou, A. Vaknin-Dembinsky, B. Gowda-Kurkalli, J.M. Gomori, I. Kassis, et al., Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis, Arch. Neurol. 67 (2010) 1187–1194.
[96] L. Sun, K. Akiyama, H. Zhang, T. Yamaza, Y. Hou, S. Zhao, et al., Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans, Stem Cells 27 (2009) 1421–1432.
[97] C. Capelli, M. Domenghini, G. Borleri, P. Bellavita, R. Poma, A. Carobbio, et al., Human platelet lysate allows expansion and clinical grade production of mesenchymal stromal cells from small samples of bone marrow aspirates or marrow filter washouts, Bone Marrow Transplant. 40 (2007) 785–791.
[98] A.F. Wise, S.D. Ricardo, Mesenchymal stem cells in kidney inflammation and repair, Nephrology (Carlton) 17 (2012) 1–10.
[99] M.B. Herrera, B. Bussolati, S. Bruno, L. Morando, G. Mauriello-Romanazzi, F. Sanavio, et al., Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury, Kidney Int. 72 (2007) 430–441.
[100] G. Raicevic, R. Rouas, M. Najar, P. Stordeur, H.I. Boufker, D. Bron, et al., Inflammation modifies the pattern and the function of Toll-like receptors expressed by human mesenchymal stromal cells, Hum. Immunol. 71 (2010) 235–244.
[101] R. Romieu-Mourez, M. Francois, M.N. Boivin, M. Bouchentouf, D.E. Spaner, J. Galipeau, Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype, J. Immunol. 182 (2009) 7963–7973.
[102] O. DelaRosa, E. Lombardo, Modulation of adult mesenchymal stem cells activity by toll-like receptors: implications on therapeutic potential, Mediators Inflamm. 2010 (2010) 865601.
[103] R.S. Waterman, S.L. Tomchuck, S.L. Henkle, A.M. Betancourt, A new mesenchymal stem cell [MSC] paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype, PLoS One 5 (2010) e10088.
[104] S. Brandau, M. Jakob, H. Hemeda, K. Bruderek, S. Janeschik, F. Bootz, et al., Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge, J. Leukoc. Biol. 88 (2010) 1005–1015.
[105] L. Raffaghello, G. Bianchi, M. Bertolotto, F. Montecucco, A. Busca, F. Dallegri, et al., Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for

neutrophil preservation in the bone marrow niche, Stem Cells 26 (2008) 151–162.
[106] M.A. Cassatella, F. Mosna, A. Micheletti, V. Lisi, N. Tamassia, C. Cont, et al., Toll-like receptor-3-activated human mesenchymal stromal cells significantly prolong the survival and function of neutrophils, Stem Cells 29 (2011) 1001–1011.
[107] L.A. Marquez-Curtis, A. Janowska-Wieczorek, Enhancing the migration ability of mesenchymal stromal cells by targeting the SDF-1/CXCR4 axis, Biomed. Res. Int. 561098 (2013) 2013.
[108] J.D. Fontenot, J.P. Rasmussen, M.A. Gavin, A.Y. Rudensky, A function for interleukin 2 in Foxp3-expressing regulatory T cells, Nat. Immunol. 6 (2005) 1142–1151.
[109] J.A. Bluestone, W. Liu, J.M. Yabu, Z.G. Laszik, A. Putnam, M. Belingheri, et al., The effect of costimulatory and interleukin 2 receptor blockade on regulatory T cells in renal transplantation, Am. J. Transplant. 8 (2008) 2086–2096.
[110] N. Perico, F. Casiraghi, E. Gotti, M. Introna, M. Todeschini, R.A. Cavinato, et al., Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation, Transpl. Int. 26 (2013) 867–878.
[111] G. Gentile, C. Somma, A. Gennarini, D. Mastroluca, G. Rota, F. Lacanna, et al., Low-dose RATG with or without basiliximab in renal transplantation: a matched-cohort observational study, Am. J. Nephrol. 41 (2015) 16–27.
[112] M.G. de Goer, E. de Herve, H. Gonzales, J.L. Hendel-Chavez, O. Decline, K. Abbed Mourier, et al., CD25 appears non essential for human peripheral T(reg) maintenance in vivo, PLoS One 30 (2010) e11784.
[113] F.W. Vondran, K. Timrott, J. Tross, S. Kollrich, A. Schwarz, F. Lehner, et al., Impact of Basiliximab on regulatory T-cells early after kidney transplantation: down-regulation of CD25 by receptor modulation, Transpl. Int. 23 (2010) 514–523.
[114] Z. Wang, B.Y. Shi, Y.Y. Qian, M. Cai, Q. Wang, Short-term anti-CD25 monoclonal antibody administration down-regulated CD25 expression without eliminating the neogenetic functional regulatory T cells in kidney transplantation, Clin. Exp. Immunol. 155 (2009) 496–503.
[115] K.A. Newell, A. Asare, A.D. Kirk, T.D. Gisler, K. Bourcier, M. Suthanthiran, et al., Identification of a B cell signature associated with renal transplant tolerance in humans, J. Clin. Invest. 120 (2010) 1836–1847.
[116] K.A. Newell, A. Asare, I. Sanz, C. Wei, A. Rosenberg, Z. Gao, et al., Longitudinal studies of a B cell-derived signature of tolerance in renal transplant recipients, Am. J. Transplant. 15 (2015) 2908–2920.
[117] C. Mudrabettu, V. Kumar, A. Rakha, A.K. Yadav, R. Ramachandran, D.B. Kanwar, et al., Safety and efficacy of autologous mesenchymal stromal cells transplantation in patients undergoing living donor kidney transplantation: a pilot study, Nephrology (Carlton) 20 (2015) 25–33.
[118] J. Tan, W. Wu, X. Xu, L. Liao, F. Zheng, S. Messinger, et al., Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial, JAMA 307 (2012) 1169–1177.
[119] G.H. Pan, Z. Chen, L. Xu, J.H. Zhu, P. Xiang, J.J. Ma, et al., Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study, Oncotarget 7 (2016) 12089–12101.
[120] Y. Peng, M. Ke, L. Xu, L. Liu, X. Chen, W. Xia, et al., Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study, Transplantation 95 (2013) 161–168.
[121] M.E. Reinders, J.W. de Fijter, H. Roelofs, I.M. Bajema, D.K. de Vries, A.F. Schaapherder, 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 (2013) 107–111.
[122] Y. Soeder, M. Loss, C.L. Johnson, J.A. Hutchinson, J. Haarer, N. Ahrens, et al., First-in-human case study: multipotent adult progenitor cells for immunomodulation after liver transplantation, Stem Cells Transl. Med. 4 (2015) 899–904.
[123] O. Detry, M. Vandermeulen, M.H. Delbouille, J. Somja, N. Bletard, A. Briquet, et al., Infusion of mesenchymal stromal cells after deceased liver transplantation: a phase I-II, open-label, clinical study, J. Hepatol. 67 (2017) 47–55.
[124] G. Remuzzi, J.S. Bromberg, Mesenchymal stromal cells: what's in the name? (and For What?), Am. J. Transplant. 13 (2013) 1625.
[125] N. Ketterl, G. Brachtl, C. Schuh, K. Bieback, K. Schallmoser, A. Reinisch, et al., A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radio-resistance, Stem Cell Res. Ther. 6 (2015) 236.
[126] M.D. Griffin, S.J. Elliman, E. Cahill, K. English, R. Ceredig, T. Ritter, Concise review: adult mesenchymal stromal cell therapy for inflammatory diseases: how well are we joining the dots? Stem Cells 31 (2013) 2033–2041.