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REVIEW

Cellular therapies in organ transplantation

Martin J. Hoogduijn[1], Fadi Issa[2], Federica Casiraghi[3] & Marlies E. J. Reinders[1]

1 Nephrology and Transplantation, Department of Internal Medicine, Erasmus University Medical Center, Erasmus Medical Center, Rotterdam, The Netherlands
2 Transplantation Research and Immunology Group, Nuffield Department of Surgical Sciences, John Radcliffe Hospital, University of Oxford, Oxford, UK
3 Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy

Correspondence
Dr. Martin J. Hoogduijn, Erasmus University Medical Center – Internal Medicine, Dr. Molewaterplein 50, Rotterdam 3015 CA, The Netherlands.
Tel.: +31107035418;
e-mail: m.hoogduijn@erasmusmc.nl

SUMMARY

Cellular therapy is a promising tool for improving the outcome of organ transplantation. Various cell types with different immunoregulatory and regenerative properties may find application for specific transplant rejection or injury-related indications. The current era is crucial for the development of cellular therapies. Preclinical models have demonstrated the feasibility of efficacious cell therapy in transplantation, early clinical trials have shown safety of several of these therapies, and the first steps towards efficacy studies in humans have been made. In this review, we address the current state of the art of cellular therapies in clinical transplantation and discuss monitoring tools and endpoints for these studies.

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Introduction

The implementation of calcineurin-inhibiting drugs with immune cell proliferation inhibitors in clinical transplantation practice in the 1980s and 1990s has greatly advanced the outcome of organ transplantation [1–3]. In particular, short-term graft survival improved dramatically after the introduction of these drugs. However, long-term graft survival did not see the same improvement, and furthermore, long-term use of immunosuppressive drugs has been indicated to lead to chronic deterioration of graft function, in particular of the kidney [4]. Therefore, there is a need for alternative therapies that are capable of improving long-term graft survival without side effects that can be used in conjunction with or even replace conventional therapy. In this perspective, cellular therapy is of major interest. Medicines based on cells may possess these properties. Different than immunosuppressive drugs, a single administration of cells could potentially have long-term effects, and while cells may have infusion-related adverse effects, there is so far little evidence for long-term toxicity effects. There are multiple cell types with immunomodulatory properties, and there are cell types that have in addition the potential to stimulate regenerative processes. Not all cell types are suitable for therapy development. Therapeutic cells need to be able to expand *in vitro* unless therapeutic amounts of cells can be harvested from a donor, and survive cryopreservation when applied, and they should allow safe routes of administration. When considering allogeneic cell therapies, the immunogenicity of the cells becomes a relevant issue.

A number of cell types possess suitable properties for the development of therapies and have been studied for their applicability and efficacy in organ transplantation. These include mesenchymal stromal cells (MSC), regulatory T cells, regulatory macrophages and tolerogenic

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dendritic cells, which are mainly studied for their immunoregulatory properties. Functional cell types, such as hepatocytes, may find use for replacement of nonfunctional tissue cells. Potentially, cell replacement can substitute organ transplantation although there are challenges with engraftment of functional cell types. It has been demonstrated that radiation preconditioning of the liver may improve the engraftment of hepatocytes [5]. The organ transplantation field may draw inspiration from studies in Duchenne syndrome, which explore the replacement of satellite stem cells in the muscle with gene-corrected induced pluripotent stem cells (iPSCs) differentiated into satellite stem cells [6]. In organ transplantation, regenerative cell therapy is mostly aimed at activation of resident progenitor cells. Therapeutic cells actively secrete regenerative compounds and furthermore release vesicles that are loaded with proteins and RNA, which may themselves be used as a form of cell-derived therapy. Cells can also be used to generate implantable bio-engineered tissues and organoids *in vitro*. Using cell reprogramming techniques and by mimicking embryological conditions in a culture dish, remarkable differentiated organoids can be generated that resemble kidney [7], liver [8], intestine [9] and other transplantable organs. These organoids find use for disease modelling and drug testing and eventually may be used for replacement of nonfunctional tissue. There is excellent literature on this topic [7,10,11]. The present review focuses on cellular therapies that can be administered to modulate alloimmune responses and initiate transplant organ regenerative processes in the transplant patient.

Mesenchymal stromal cell therapies

The first studies with MSC in clinical transplantation

Mesenchymal stromal cell are the most studied clinical cellular therapy in the field of organ transplantation so far. MSC are a heterogeneous population of multipotent cells usually obtained after ex vivo expansion of bone marrow (BM), adipose tissue and umbilical cord (UC). In the last decades, MSC have raised the interest of transplant immunologists because they display unique immunomodulatory activities. In several preclinical models of transplantation, MSC prolonged graft survival and induced tolerance to skin [12], heart [13,14], kidney [15,16], islet [17] and corneal allografts [18,19]. Although short-lived after intravenous infusion [20], MSC promote long-term immunomodulation by conferring a pro-tolerogenic phenotype to regulatory T cells, tolerogenic antigen-presenting cells (APC) and M2 macrophages [14,17,18,21].

Mesenchymal stromal cell immunomodulatory properties highly depend on the microenvironment they encounter upon administration. Indeed, MSC exposed to particular inflammatory signals can acquire an opposite function, promoting inflammation [16,22] and acting as APC following MHC-II upregulation [23]. One of the major determinants of the effect of MSC is the timing of administration [16,22]. It appears from preclinical models that pretransplant infusion of MSC prolongs allograft survival, whereas infusion within days after transplantation promotes alloreactivity [16].

Phase I clinical studies, primarily aimed at assessing safety and feasibility of MSC, have been conducted in kidney [24–28], liver [29–31], lung [32,33] and small-bowel [34,35] transplantation. In all studies, MSC, isolated either from autologous [24–28,34] or allogeneic [30,32,33] BM or from UC [29,31], demonstrated an exceptional safety profile. Administration of 1–2 $\times$ 10^6 autologous BM-MSC/kg was first performed in two living-donor kidney transplant patients seven days after transplantation [25]. Unexpectedly, both patients developed transient acute graft insufficiency. After amendment of the protocol, the two subsequent patients received BM-MSC the day before transplantation and no longer experienced engraftment syndrome [24]. At 5- to 7-year follow-up, both patients maintained stable graft function [28] and one recipient developed a long-lasting immune profile characterized by an increased regulatory T-cell/memory $CD8^+$ T-cell ratio. Increased regulatory T-cell expansion was also observed in living-donor kidney transplant recipients receiving double intravenous injections of autologous BM-MSC one day before and 30 days post-transplant [27]. A study in six living-donor kidney transplant patients employed MSC therapy as a treatment for subclinical rejection and interstitial fibrosis/tubular atrophy (IF/TA) [26]. Patients received two intravenous infusions of autologous BM-MSC, 7 days apart. Surveillance biopsies performed in two MSC-treated recipients after MSC infusion showed complete resolution of subacute cellular rejection (tubulitis) and IF/TA, suggesting that MSC could protect the kidney graft from chronic damage [26].

In liver transplantation, a prospective, controlled phase I study showed safety and feasibility of a single post-transplant intravenous injection of 1.5–3 $\times$ 10^6/kg BM-MSC derived from a third-party donor. Rejection rates, graft survival, histological findings on 6-month protocol biopsies and Treg frequency in the peripheral

blood during the 12-month follow-up were comparable to control liver transplant patients [30]. Attempts to wean immunosuppression failed in all but one patient [30]. In the study by Shi *et al.* [29], liver transplant recipients with biopsy-proven acute rejection receiving a single intravenous infusion of 1×10^6/kg UC-MSC showed a higher decrease in liver enzymes compared with the control group receiving standard immunosuppression. In addition, increased circulating regulatory T-cell frequencies and plasma levels of the immunoregulatory molecules TGF-beta and prostaglandin E_2 (PGE_2) were detected in MSC-treated patients [29]. A study in which UC-MSC were administered to 12 liver transplant recipients with biliary complications at 1, 2, 4, 8 and 16 weeks after recruitment reported a significantly lower need for clinical interventions and a higher 1-year graft survival in MSC-treated patients compared with controls [31].

In lung transplantation, a number of studies have been conducted using allogeneic BM-MSC for ameliorating chronic lung allograft dysfunction. Chambers *et al.* [32] reported a minor and transient fall in mean arterial pressure and O_2 saturation in patients with chronic lung allograft dysfunction after injection of allogeneic BM-MSC. Compared with baseline, MSC-treated patients showed a trend towards a slower decline in forced expiratory volume after 1 year. A mild beneficial effect of MSC on lung function was also reported by Keller *et al.* [33]. A study in nine recipients with moderate bronchiolitis obliterans syndrome (BOS) refractory to standard therapy demonstrated no significant alterations in pulmonary function 24 h, 1 week and 1 month after a single infusion of 1, 2 or 4 million BM-MSC/kg [33]. At 1-year follow-up, five patients exhibited a stabilization of lung function and three patients showed a lesser rate of functional decline than prior to MSC infusion. Patients given the lowest MSC dose showed an increase in the frequency of Tregs and a favourable pro-inflammatory/anti-inflammatory plasma cytokine profile.

Finally, MSC have been tested in a small number of patients undergoing small-bowel transplantation. A case report described a patient with severe, refractory bowel graft dysfunction after intestinal transplantation who showed a rapid improvement in clinical parameters and histological evidence of marked focal regenerative changes after treatment with MSC [35]. In an additional study, six patients underwent intestinal transplantation and received 3 doses of autologous BM-MSC [34]. The first dose of MSC was administered in the donor intestinal artery during the transplant procedure, while the second dose and third dose were injected into the mesenteric artery 15 and 30 days post-transplant, with no adverse effects.

These early studies demonstrated that MSC therapy is safe and feasible in transplant patients, and evoked interest in studies to the therapeutic effects of MSC treatment in organ transplantation.

Towards phase 2–3 trials

The step from safety/feasibility studies towards phase 2–3 trials slowly progressed over the last years. This may be due to the fact that results of the early studies had to be awaited, which seems wise, as proven safety and feasibility are required for scaling up. Of interest, a lot has been learned from individual case studies [24,25], which helps the development of future studies. As mentioned above, it was demonstrated that timing of MSC infusion was of importance as an engraftment syndrome with infiltration of immune cells and C3 deposits were found when MSC were administered at 7 days after kidney transplantation, which was not observed when MSC were given before transplantation [24]. Moreover, an interesting case provided evidence that in a renal transplant recipient, infusion of autologous bone marrow MSC was associated with safe complete discontinuation of maintenance immunosuppression after transplantation allowing a state of immune tolerance [36]. Progression of the field is also influenced by logistic and regulatory issues, which accompany cell-based therapy such as clinical grade cell production facilities and associated costs. As funding and equipment are lacking, it is obvious that academic centres need support from a commercial partner [37].

So far, there are few randomized controlled studies with MSC although reference groups or whole cohorts were included for comparability. In a phase 1–2 study by Erpicum *et al.*, the 1-year follow-up of a single infusion of third-party MSC post-kidney transplantation in addition to standard immunosuppression was reported. This therapy was safe and associated with a transient increase in regulatory T cells at day 30. It furthermore improved early allograft function compared with the control group and whole cohort [38]. Incidences of opportunistic infections and acute rejection were similar in the MSC group compared with controls. In this study, four MSC-treated patients developed antibodies against MSC or shared kidney-MSC HLA; however, renal function remained stable leaving the clinical relevance of this alloimmunization unclear. The development of anti-HLA antibodies was not reported in a

recent study where HLA selected allogeneic MSC were infused with low-dose tacrolimus [39]. This design was proven to be safe with a follow-up of 1 year after transplantation [40]. In this study, no major alterations in T- and B-cell populations or plasma cytokines were observed upon MSC infusions.

The study by Tan *et al.* is the largest clinical trial with MSC in the transplant setting so far. In a randomized controlled trial, it was demonstrated that treatment with autologous BM-MSC, infused at day 0 and day 14 after transplantation, was safe and feasible as induction therapy and allowed for calcineurin inhibitor reduction [41]. In this study, immune monitoring was not performed. The capability of MSC to allow reduction of calcineurin inhibitors has also taken up by other groups. In a study in living kidney transplantation with third-party MSC (5×10^6/kg body weight at day 0 and 2×10^6/kg body weight at day 30) and a control group, infusion of MSC was safe and allowed for a 50% reduction of calcineurin inhibitors. In this study, there was no difference in circulating lymphocytes and in donor-specific T-cell proliferation between the MSC group and control group [42,43]. Most studies so far focused on BM-MSC. A prospective multicentre randomized trial in which MSC were intravenously infused at day −1 (2×10^6/kg body weight) and administered via the renal artery during the kidney transplantation procedure (5×10^6/kg body weight) in 21 patients vs. 21 controls was performed with umbilical cord-derived MSC. This study reported no difference in the incidence of delayed graft function and acute rejection between the MSC group and control group, and estimated glomerular filtration rates were similar between the two groups [44]. There were no adverse clinical effects of MSC administration. In this study, immune monitoring results were not presented.

A recent phase 2–3 study recruited 70 patients in the period 2014–2020 to test the hypothesis that MSC in combination with the immunosuppressive everolimus facilitates early withdrawal (at 8 weeks) of tacrolimus with the aim to preserve renal function and structure. The primary endpoint is fibrosis measured by quantitative staining of Sirius Red. Secondary endpoints include adverse events, including infections, renal function and immune monitoring. Results are expected soon [45].

Interesting directions for future clinical trials with MSC after renal transplantation include the timing and frequency of MSC injections with the aim to limit fibrosis and alloimmune responses, to allow calcineurin inhibitor withdrawal and probably induce a tolerogenic state. Moreover, MSC infusion during organ preservation may participate in limiting damage to the graft [46].

Regulatory T cells

$CD4^+CD25^+$ regulatory T cells (Tregs) were discovered over 20 years ago, and following the identification of their master transcription factor, FOXP3 has become central to major therapeutic developments in the fields of autoimmunity, transplantation and cancer. There is evidence for the existence of thymic Tregs (tTregs) in bony fish some 400 million years ago [47], with peripherally induced Tregs (pTregs) following in placental mammals where 'on-demand' regulation was required to protect the foetus [48]. The vast array of Treg suppressive mechanisms that have been identified may be linked to the need for redundancy in the system [49]. This could be due to the need to control different cell types through cell-specific mechanisms, or the many environments in which Tregs are active [50]. However, it is also possible that some of these identified mechanisms are an artefact of the experimental system used to investigate Treg activity, with *in vitro* suppression assays highlighting effects such as the CD25/IL-2 consumption phenomenon, that may not be as relevant *in vivo* [51]. Moreover, the ability to abrogate Treg function through the deletion/blockade of specific genes or molecules may, in fact, be a reflection of how easy it is to damage a finely balanced system – removal of a single wheel from a mechanical watch will break it – rather than necessarily highlighting the functional importance of these molecules.

Challenges in our understanding of the biology of Tregs aside, these cells have enjoyed an accelerated clinical development leading from mouse studies to phase II trials in transplantation within only a few years (reviewed in Ref. [52]). The two principal clinical approaches are to infuse autologous polyclonal ex vivo-expanded Tregs, or to induce their expansion/generation with the use of low-dose or mutein IL-2 treatment. Clinical IL-2 therapy is largely being investigated in autoimmunity [53], while in transplantation, adoptive Treg therapy is more advanced (although there is now a revival of interest in IL-2 treatment, and particularly combined IL-2/Treg treatment, in transplantation [54–56]). Enthusiasm for Tregs stems from the potential advantage of modifying the balance between effector and regulatory cells towards a state, which is more permissive to partial immunosuppression withdrawal or discontinuation [57]. Published data from Treg cell therapy trials in transplantation provide some cause for

cautious optimism, with evidence for safety and perhaps a reduced requirement for induction immunosuppression in renal transplantation [58] or even maintenance immunosuppression in liver transplantation [59]. A recent study in 11 kidney transplant patients demonstrated that stable monotherapy immunosuppression was achieved in 8 patients receiving autologous Treg [60]. While these trials are still in early phases, the benefits of reducing immunosuppression are becoming apparent in terms of lower viral infection rates and normalization of immune composition [58]. Encouragingly, despite the wide variety of techniques being used to produce these adoptive Treg cell therapies (e.g. [61–63]) and the anxiety regarding Treg stability and cell product purity, no detrimental effects of infusion have yet been detected, although the small number of patients treated with Treg so far cannot rule out this possibility completely. Increased alloantibody responses observed in lymphodepleted nonhuman primate heart allograft recipients after infusion of Tregs shortly after transplantation [64] and the report of the development of fever and transient neutropenia, lymphopenia and mild liver graft dysfunction in a patient after Treg administration [65] demonstrate that safety of Treg therapy has to be monitored at all times.

In the light of the excellent short-term results after transplantation, later phase trials will need to be designed carefully to ascertain whether Tregs are truly effective [66,67]. Immune monitoring data are therefore critical for identifying subtle changes in immune composition that may not manifest in early clinical outcomes [68]. The wealth in genetic and cellular data related to transplant rejection and regulation that have been collected over decades will form an important basis for identifying such changes, through technologies that can be standardized across centres [69–72].

Next-generation Treg therapeutics are now focused on antigen specificity [73], with chimeric antigen receptor (CAR) Tregs taking centre stage [74]. These cell products allow for intricate modification in antigen recognition, costimulation, and signalling domains, theoretically providing greater control of the desired effects [75,76]. Trials of CAR Tregs are planned by a number of commercial enterprises; therefore, the precise details of these studies are not publicly available. Nevertheless, while enthusiasm is justified, it is not yet entirely clear whether CAR Tregs are indeed effective in humans (or whether they will be active against memory responses [77]). Moreover, their production is further complicated by the need for complex genetic modification [78], making polyclonal Tregs an attractively simple proposition if their efficacy is confirmed. Nonetheless, as with many cellular therapies, a significant challenge remains in the production capacity/capability of Treg therapeutics. As it stands, production is costly, is time-intensive and requires substantial operator input [79]. Methodologies that address these challenges while maintaining quality are of significant value. Research in this aspect of production will be critical over coming years in order to ensure Treg therapy can be viably adopted into clinical transplantation practice.

Regulatory myeloid cells

Dendritic cells and macrophages are diverse in function and contain a variety of subsets with different phenotypical and functional characteristics that possess immune regulatory properties. Regulatory macrophages comprise a subset of macrophages that is induced upon stimulation of activated macrophages with a variety of stimuli [80]. It has been described that Fcγ receptor stimulation on mouse Toll-like receptor-activated macrophages induces these cells to produce immune suppressive IL-10 rather than immune-activating IL-12, and induces $CD4^+$ T cells to produce IL-4 [81]. The induction of regulatory macrophages that show increased anti-inflammatory cytokine production in combination with reduced pro-inflammatory cytokine production has also been demonstrated upon costimulation of activated macrophages with a wide variety of other factors such as PGE_2 in mouse macrophages [82] and TGF-β [83] and IFN-γ in human macrophages [84]. The induction of regulatory properties in macrophages after phagocytosis of apoptotic cells is a mechanism that is seen across species [85]. Regulatory macrophages thus represent a family of macrophages that has in common their role in controlling immune responses and contribution to tissue homeostasis. Similarly, regulatory dendritic cells, known as tolerogenic dendritic cells in the transplantation field, are a subset of dendritic cells that act in a variety of ways to promote transplant tolerance, nicely summarized by Ochando *et al.* [86].

The tissue protective immune controlling property of regulatory macrophages and dendritic cells make them of interest for cellular therapy in organ transplantation. Several studies have reported graft survival-promoting or even tolerance-inducing effects of donor-derived tolerogenic dendritic cells in murine models [87–89]. A type of regulatory macrophage induced by stimulation of peripheral blood monocytes by macrophage colony-stimulating factor (M-CSF) and interferon-γ (IFNγ)

prolonged allograft survival by 24 days in a mouse heart transplant model [90]. Like the tolerogenic dendritic cells, these regulatory macrophages were of donor origin, and recipient or 3rd-party regulatory macrophages given 8 days before transplantation were not effective in this model. A similar type of regulatory macrophage has been suggested to be an effective suppressor of the xenoimmune response [91]. Conde *et al.* [92] demonstrated that CD40-CD40L blockade induces DC-SIGN-expressing regulatory macrophages that are capable of prolonging heart allograft survival.

The promising results from *in vitro* and preclinical studies have led to the translation of these studies to clinical trials. Early clinical experience with regulatory macrophages in organ transplant patients stems from a decade ago, when two living-donor kidney transplant patients received donor-derived regulatory macrophages a week prior to transplantation, which were induced by stimulating human monocyte-derived macrophages with IFN-γ for 18–24 h [93]. The patients tolerated the cells well and underwent kidney transplantation without complications. There were no signs of rejection in the first year after cell infusion. In follow-up studies, it was demonstrated that kidney transplant patients who received 2.5–7.5 × 10^6 regulatory macrophages seven days before kidney transplantation showed elevated levels of $TIGIT^+FOXP3^+$ regulatory T-cell subtype [94]. In one patient, $TIGIT^+FOXP3^+$ regulatory T-cell levels were elevated seven years after transplantation. Tolerogenic dendritic cells have also been introduced to the clinic in the first phase 1/2 clinical trials [95]. In the recently published ONE Study, living-donor kidney transplant patients were treated with regulatory macrophages, and autologous tolerogenic dendritic or regulatory T cells [58]. Patients in the different cellular therapy groups received the same immunosuppressive regimen and were grouped and compared with a reference group. In the cell therapy group, basiliximab induction was omitted and mycophenolate mofetil tapering was allowed. The replacement of basiliximab by cell therapy did not result in elevated acute rejection rates or adverse clinical events. The cell therapy group as a whole showed a lower infection rate compared with the reference group. Similar to MSC and Tregs, there are hints for therapeutic efficacy of regulatory myeloid cells in organ transplantation, which needs further exploration in large controlled trials.

Other cell types and extracellular vesicles

In addition to immunomodulatory purposes, cellular therapies in organ transplantation may also be applied to replace functional cells in diverse organs, such as hepatocytes, podocytes, tubular cells or alveolar cells. Strategies to replace lost or injured cells by culture-expanded therapeutic cells are complex because of accessibility issues, poor *in vitro* proliferation of functional cells and limited survival of exogenous cells after administration. *Ex vivo* organ perfusion techniques may offer a solution to some of these problems, as discussed by Hosgood *et al.* in this focus issue. Furthermore, extracellular vesicles may represent an alternative for some aspects of cellular therapies. Extracellular vesicles mimic some of the functional properties of cells, while they behave differently with respect to biodistribution and have no survival issues. Extracellular vesicles contain a variety of molecules with regeneration-inducing and immunomodulatory function, including proteins, lipids, μRNAs and mRNAs [96]. Furthermore, the membranes of extracellular vesicles contain membrane-spanning proteins, including HLA, that also play a role in the biological function of vesicles. It has been proposed that extracellular vesicles are regulators of immune responses [96] and it has been demonstrated that administration of donor dendritic cell-derived vesicles prior to transplantation prolongs heart allograft survival in a murine model [97].

Mesenchymal stromal cell are potent secretors of extracellular vesicles [98]. MSC-derived vesicles have been indicated to possess immune regulatory properties [99], prolong graft survival in vascularized composite allotransplantation [100] and stimulate angiogenic processes [101]. Therefore, extracellular vesicles isolated from conditioned medium of cultured MSC may be used for therapy development. One of the challenges would be to isolate these vesicles free from contaminating soluble proteins, as these accumulate in the same fractions as vesicles using conventional centrifugation and filtration techniques [102]. Currently, extracellular vesicles have not been examined in the context of clinical trials, although a number of studies have examined the effect of vesicles on isolated animal and human organs, demonstrating a potential reparative effect of vesicles [103]. In addition to collecting extracellular vesicles from cell culture supernatants, it is possible to generate vesicles from the membranes of MSC or other cell types. These vesicles can be generated in large numbers free from contamination by soluble proteins, and interact with cells of the immune system [104]. They may therefore represent an up-scalable alternative to extracellular vesicles.

Table 1. Overview of the main safety and efficacy outcomes of the major cell types studied in clinical trials in transplant patients.

Cell type	Safety aspects	Efficacy outcomes
Mesenchymal stromal cells	No adverse effects in majority of patients	Indication for regulatory T-cell expansion
	Engraftment syndrome reported in 2 patients after MSC infusion 7 days post-transplantation	Indication that MSC treatment allows weaning of immunosuppressive drugs
	Possible formation of antibodies against allogeneic MSC	No increase or reduced incidence of opportunistic infections
Regulatory T cells	Evidence for safety, with report of lymphopenia and mild liver graft dysfunction in one patient	Hints for reduced requirement for induction immunosuppression in renal transplantation and reduced maintenance immunosuppression in liver transplantation
Regulatory myeloid cells	No evidence of adverse effects in studies published so far	Hints for regulatory T-cell expansion after regulatory macrophage administration
		Potentially allowing weaning of immunosuppressive drugs
Extracellular vesicles	No safety data yet	No clinical trials yet, but indication for reparative effects on isolated organs

A summary of the major outcomes of clinical studies in transplant patients with the major cell types is shown in Table 1.

Endpoints and monitoring of cellular therapies

A very challenging aspect of clinical trials with cellular therapy is to define endpoints that can measure safety, feasibility and efficacy accurately and to monitor the treatment. So far, trials in transplantation with cells mainly focused on feasibility and safety, although secondary endpoints were included with a focus on mechanistic insight [105]. For safety, potential risks include direct toxicity related to the cell infusion and over-immune suppression resulting in (opportunistic) infections and malignancies. These should all be accurately monitored and documented. It is advised to document the (serious) adverse events (SAE) according to MedDRA® (Medical Dictionary for Regulatory Activities), which is the international medical terminology developed under the auspices of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. This method has been used in two recently published trials with cell therapy in renal transplantation and allows for comparison between trials [40,58]. Since the development of infections and malignancies may take time, long-term follow-up of patients is required after finalizing clinical trials.

Allogeneic cells have numerous advantages compared with autologous cells. Indeed, they are directly available and allogeneic cell products can be easily standardized. However, allogeneic cells can induce alloimmune responses [38], which might increase the risk for allograft rejection and graft dysfunction. Therefore, in trials with allogeneic cells, analysis of anti-human leucocyte antigen-specific antibodies related to allogenic MSC infusions should be performed [40].

Traditional primary efficacy endpoints for novel immunosuppressants in solid organ transplantation focus on patient death, graft failure, biopsy-proven acute rejection (BPAR) and graft (dys)function (defined by criteria as measurement of creatinine/inulin clearance for kidney dysfunction). Although these endpoints have clear roles in research that aims to improve short-term clinical outcomes, inhibition of early rejection does not translate into long-term graft improvement. Moreover, graft failure is rare in the early years after transplantation, and acute rejection rates have markedly declined. In addition, trials with cellular therapy are labour-intensive and costly, and trials with conventional endpoints would need a large population, which is a great

challenge. As an example, to assess BPAR rates as primary objective, a patient population of at least 320 patients is needed to obtain a reduction of 50% in rejection rate, assuming a rejection rate of 20% in the control group with two-tailed significance of 0.05 and 80% power (chi-quadrate test), in a prospective randomized controlled trial [106]. For all these reasons, surrogate endpoints for long-term graft function are necessary. In large patient cohorts in renal transplantation, glomerular filtration rates (GFR), CKD stages, proteinuria, appearance of *dn*DSA, histology of antibody-mediated rejection, IFTA and transplant glomerulopathy are all associated with heightened risk of late graft functional decline/failure [107–109]. However, unfortunately, there is no approved surrogate marker for long-term graft function yet.

The Banff score is the standard setting for the pathologist to evaluate renal transplant biopsies [110]; however, with this score precise quantification of, for example, interstitial fibrosis is difficult since it is semi-quantitative and there is inter-observer variability [111]. In the randomized controlled Triton trial, a surrogate quantitative marker for the degree of fibrosis was used by assessing Sirius Red staining in renal biopsies, which specifically stains collagen types I and III [112]. Indeed, several studies showed that Sirius red staining can be used as an accurate and reproducible method for measuring the degree of interstitial fibrosis [113]. O'Connell *et al.* [114] developed a panel consisting of 13 genes that is highly predictive in kidney allograft biopsies for the development of fibrosis at 1 year after transplantation. Such molecular panels may be used to adjust treatment of transplant patients at an early stage.

In all trials, graft function is included as secondary endpoint. As an example, in renal transplantation the determination of renal function (GFR) is of importance for assessing safety and for follow-up after cell-based therapy. However, it is of importance to note that GFR clearly has also limitations, since early subclinical disease, which may lead to late failure, is not captured. Besides graft function, immune monitoring is crucial in the evaluation of cellular therapy. The ONE Study consortium developed a standardized method, which monitors the general immune response and T-cell, B-cell and dendritic cell subsets [70]. This method has been used in the ONE Study, as well as in studies with MSC therapy after renal transplantation [26,40,58]. In addition, functional assays, such as the *in vitro*-mixed lymphocyte reaction and measurement of cytokines, might give mechanistic insight after cell therapy [106]. Other described endpoints include cardiovascular mortality and morbidity, as MSC have also been used for cardiovascular indications and might influence coexisting disease in the transplant recipient [45].

Recently, it was shown that combining factors as composite surrogate endpoint probably better reflects the heterogeneity of graft failure compared with single-cell markers. Of interest, the iBOX score has recently been validated in different patient cohorts and has shown robustness in this respect [109]. This method has not yet been applied in cell therapy trials.

Future perspectives

Cellular therapies are a promising novel way of treating immune- and injury-related complications in organ transplant patients. Therapies with various cells types with specific properties are under investigation and may be applied for different indications. The majority of trials so far have shown safety of cellular therapies in organ transplant patients. The next important step is to show efficacy of cellular therapies. This involves up-scaling of GMP production of therapeutic cells and performing large placebo-controlled trials. Collaborations between academic centres and industry are essential to achieve this. Furthermore, better understanding of biodistribution, survival and interaction of administered cells with host cells is crucial for the development of efficacious cellular therapy. In contrast to past beliefs, exogenous cells may not have a long lifespan after administration. MSC have been shown to disappear largely within 24 h after intravenous administration and rather instruct host cells to adapt a therapeutic phenotype during their brief presence [20,115]. The study of Roemhild *et al.* [60] reported a transient increase in Treg levels with a return to control levels 12 weeks after administration of Tregs. For other cell types, survival times are not clear, and the use of autologous cells in clinical studies hampers long-term tracking.

In theory, some of these effects may be mediated via nonviable therapeutic cell-derived products, such as soluble proteins, vesicles with their membrane-bound proteins or even intact dead cells. Another direction that cellular therapies in the field of organ transplantation may move to is to treat patients at early stages of organ injury. Early treatment of inflammatory or degenerative processes may repair organs and eventually make transplantation obsolete. Results of studies in the near future will determine in which way cellular therapies will develop.

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