TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

# Regulatory T cells: first steps of clinical application in solid organ transplantation

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**Keywords**
cellular therapy, immunosuppression, regulatory T cell, transplantation.

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**Conflicts of interest**
The authors have declared no conflicts of interest.

Received: 2 December 2014
Revision requested: 26 January 2015
Accepted: 13 May 2015
Published online: 3 June 2015

doi:10.1111/tri.12608

## Summary

Solid organ transplantation is the treatment of choice for patients with end-stage organ failure. To prevent rejection of the transplanted organ continuous treatment with immunosuppressive medication is needed. Immunosuppression may be harmful to the transplant recipient, increasing the risk of cancer, infections and cardiovascular disease. To improve transplant and patient survival, there is a need for an immune-modulatory regimen that is not only potent in preventing rejection of the transplanted organ, but has less side effects compared to current immunosuppressive regimens. Increasingly, transplantation research focusses on regulatory T cell (Treg) therapy to achieve this aim, in which Treg are used as a strategy to allow reduction of immunosuppression. Currently, the first clinical trials are underway investigating the safety and feasibility of Treg therapy in renal transplantation. This review gives an overview of the rationale of using Treg therapy in transplantation, previous experience with Treg therapy in humans, and the expected safety, potential efficacy and cost-effectiveness of Treg therapy in solid organ transplantation.

## Introduction

Solid organ transplantation is the treatment of choice for patients with end-stage organ failure, increasing patient survival and improving quality of life [1,2]. To prevent rejection of the transplanted organ continuous treatment with immunosuppressive medication is needed, which may be harmful due to nephrotoxicity and vascular disease caused by calcineurin inhibitors (CNIs). Furthermore, general immunosuppression poses several risks to transplant recipients, of which an increased risk of infection and cancer are the most important [3]. These unintended effects are a barrier to patient longevity.

To improve transplant and patient survival, there is clearly a need for an immune-modulatory regimen that allows minimization of the conventional, mostly CNI-based immunosuppressive drugs. Increasingly, transplantation research focusses on cell-based therapy, in which immunological cells with regulatory characteristics are used as a strategy to allow immunosuppressive drug minimization [4].

## Regulatory T cells (Treg)

Transplant tolerance refers to the situation in which there is no graft rejection despite the absence of long-term immunosuppression as opposed to the situation where immunosuppressive drugs are simply minimized. There are a number of lymphoid cell types with regulatory capacity that can promote tolerance induction in animal models of transplantation [4]. Of these cell types, the most widely studied are a subset of CD4+ T cells; regulatory T cells (Treg) [5,6]. The ability of Treg to suppress the immune system is illustrated by its role in preventing autoimmunity. Although negative selection during T-cell ontogeny plays a fundamental role in establishing self-tolerance, the

 

importance of Treg in maintaining self-tolerance is clearly shown by both clinical observations and in animal models. For example, patients with the Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome have dysfunctional Treg and demonstrate profound autoimmunity, mainly affecting the intestines, skin and endocrine glands. Also, specific depletion of Treg in otherwise normal healthy mice results in lethal autoimmunity [7,8].

The underlying rationale for using Treg as a cell-based therapy is to move the balance from predominantly proinflammatory T cells to a T-cell population enriched with Treg aiming to minimize the risk of rejection. The most reliable way to define Treg is by function rather than by phenotype. However, for the purpose of cell-based therapy, it is necessary to use defined markers for cell isolation which allow good manufacturing practice (GMP) manipulation. CD4+ thymus-derived naturally occurring Treg (nTreg) are most readily identified as having high expression of the surface antigen CD25 (the high-affinity IL-2 receptor α-chain), the expression of a specific intracellular transcription factor Forkhead box P3 (FOXP3) and low expression of the surface antigen CD127. As FOXP3 is not a surface marker, it cannot be used to isolate nTreg. The surface expression of CD127 is inversely correlated with FOXP3 expression [9,10] and has allowed for refinement of the nTreg phenotype with studies showing that CD4 + CD25 + CD127lo cells are approximately 5 times more potent than CD4 + CD25hi cells [11]. The expression of CD45RA might also be helpful in delineating different subpopulations of human Treg, where activated Treg (CD45RA-FOXP3hi) and resting Treg (CD45RA + FOXP3lo) are suppressive [12]. It is now possible to purify nTreg from peripheral blood [13,14], expand them *ex vivo* [15–17] and inject the expanded cell product back into the patient [18]. nTreg are normally expanded in GMP facilities after depletion of CD8+ cells and enrichment of CD25+ cells followed by stimulation by beads coated with anti-CD3/anti-CD28 antibodies in the presence of recombinant human IL-2. Sirolimus can be added to the cultures to minimize the outgrowth of non-Treg. Whilst flow cytometry-based techniques allow a more complex phenotype to be defined and greater purities to be achieved, this technique is challenging, such that therapeutic Treg flow-sorting is restricted to a small handful of centres worldwide. In contrast, magnetic bead isolation provides a GMP-compliant process (closed system), and although it is limited in terms of markers (e.g. GMP purification reagents for CD127 not yet available), acceptable levels of purity are achieved [19].

The expansion protocol described above generates polyclonally expanded nTreg, as opposed to alloantigen-driven iTreg for which allogenic antigen-presenting cells are used. On a per-cell basis, alloantigen-driven iTreg may be more potent than polyclonally expanded nTreg and may offer advantages in terms of specificity, but to date there have only been a limited number of studies in which nTreg and iTreg have been compared directly. iTreg generated by stimulation with allogenic dendritic cells were shown to have increased potency compared with polyclonally expanded nTreg in a humanized skin graft model. Furthermore, iTreg generated by stimulation in the presence of TGF-β appeared to have similar potency to nTreg in a xenogeneic mouse model of graft-versus-host disease (GvHD) [20,21]. There are, however, concerns about the stability of iTreg, with reports showing loss of FOXP3 expression [22], which can result in conversion of iTreg into effector T cells. The mechanisms of immunosuppression by Treg is beyond the scope of this review, and we recommend the review by Vignali *et al.* [23].

## Experience with Treg in animal transplantation models

Most of the preclinical studies showing that cellular therapy with Treg can control allograft rejection in the mouse used expanded alloantigen-driven Treg as opposed to expanded polyclonal nTreg, mainly because *mouse* nTreg expand far less readily than their human counterparts. However, *mouse* nTreg can control GvHD [17] and limited studies have shown that expanded *mouse* nTreg can control allograft rejection [24], although perhaps not as efficiently as alloantigen-driven Treg [25]. On the basis of these and other studies and the fact that human nTreg can be expanded by at least 100-fold, our group demonstrated that expanded *human* nTreg can control the rejection of *human* allografts in a humanized mouse model [11,26], helping to support the concept of using expanded nTreg as cellular therapy in the ONE study. As expansion of alloantigen-driven Treg may lead to a cell product that is more potent [27–29], these Treg are also investigated in the ONE study.

## Experience with Treg in humans

To date, Treg have been used in solid organ transplantation in only one clinical trial, and although this remains unpublished [30], expanded nTreg have been infused as a treatment in patients with GvHD [18] and as a GvHD prophylaxis in patients receiving haematopoietic stem cell transplantation [31,32]. Although these studies were too small to show efficacy, they did show that infusion of expanded nTreg was safe. Treg have also been administered in 10 paediatric patients with new-onset type 1 diabetes, in which no toxicity was noted [33]. The published studies that have applied Treg therapy in humans are summarized in Table 1.


 

**Table 1.** Published studies that have investigated Treg therapy in humans.

| Disease | Number of patients | Total number of Treg infused | Main results | References |
|---|---|---|---|---|
| GvHD after HSCT | 2 | $1 \times 10^5 - 3 \times 10^6$/kg bw | 1. Discontinuation of immunosuppression in patient with chronic GvHD<br>2. Slowing of pace of clinical deterioration in patient with acute GvHD | [18] |
| Prevention of GvHD after HSCT | 23 | $1–30 \times 10^5$/kg bw | Reduction in acute GvHD (grade 1–2) compared with historical controls | [31] |
| Prevention of GvHD after HSCT | 28 | $2–4 \times 10^6$/kg bw (unexpanded) | 1. Acute GvHD (≥ grade 2) in only two of 28 patients<br>2. No chronic GvHD | [32] |
| Early onset Type 1 DM | 10 | $10–20 \times 10^6$/kg bw | 1. Less insulin requirement in treated versus non-treated patients<br>2. Higher C-peptide levels in treated versus non-treated patients | [33] |

Bw, bodyweight; DM, diabetes mellitus; GvHD, graft-versus-host disease; HSCT, haematopoietic stem cell transplantation.

## Treg in solid organ transplantation

A Japanese group undertook a clinical trial of donor antigen-driven Treg in 10 patients undergoing living-donor liver transplantation [30]. They found that Treg therapy was safe and that it allowed cessation of immunosuppression in six patients. However, the patients in this study were splenectomized during the operation and received cyclophosphamide before the cell infusion. Furthermore, minimization of conventional immunosuppression without graft loss in liver transplantation is common, and therefore, it is difficult to determine to what extent Treg therapy contributed to this outcome.

The ONE study is a multicentre phase I/IIa clinical trial in which cellular therapy is investigated in *renal* transplantation, and recruitment of the first patients in the cell-based therapy arm of the study began in the summer of 2014 (www.onestudy.org) [34]. In two UK centres and a centre in Berlin, autologous *ex vivo* expanded nTreg will be administered to recipients of living-donor renal transplants whilst in centres in Boston and San Francisco, alloantigen-driven Treg will be administered to similar patients. In these five centres, the cells will be administered typically 3–5 days post-transplant. Other cell types that will be investigated in the ONE study are T regulatory type 1 cells, tolerogenic dendritic cells and regulatory macrophages. The main aim of this study will be to investigate the feasibility of GMP manufacture of regulatory immune cells for clinical use and the safety of cell infusion. The study will examine biopsy-proven rejection episodes as the primary clinical endpoint during a follow-up of 60 weeks after transplantation. Data from this group will be compared to a reference group in which the patients have received standard immunosuppressive treatment consisting of induction with a monoclonal IL-2 receptor antibody (basiliximab) and initial maintenance treatment with tacrolimus, mycophenolate mofetil and prednisolone [35]. Patients in the cell therapy arm will not receive induction therapy, but will get the same maintenance treatment as in the reference group in lower doses. Although this is a small study (recruitment aim is 56 patients in total), the most important aspect of the ONE study is that it represents the start of cellular therapy in solid organ transplantation. Lessons are being learned by the clinical and scientific teams involved in the trial and perhaps more importantly, by the various national regulatory agencies. There is no doubt that these lessons will help shape larger trials that will follow.

## Dosing of Treg

The fact that safety is the major emphasis in the initial trials means that most will follow a dose-escalation format. Doses in published trials in type 1 diabetes mellitus and haematopoetic stem cell transplantation have been between $0.1 \times 10^6$ and $20 \times 10^6$/kg of bodyweight with no adverse events [31,33]. The dose of Treg that will give the optimal immunosuppression in solid organ transplantation is not known. The doses used in trials designed to look for therapeutic effect will to some extent be a pragmatic compromise between doses shown to be safe and the cell number that can be reliably generated in GMP expansion protocols. In comparing the doses used in previous trials, it is important to keep in mind that the optimal dose will depend on whether Treg are investigated in immune-deficient or immune-competent patients, with a further distinction between immune-competent patients who will or will not receive treatment with conventional immunosuppression. This is illustrated by the higher Treg dose used in trials investigating patients with type 1 diabetes mellitus [33] compared to patients who underwent haematopoietic stem cell transplantation [31]. However, other groups suggest that the optimal dose might be as high as $3–5 \times 10^9$ [36].

Another question to which as yet there is no answer is when is the optimum time for Treg administration relative

to the time of transplant. This in part will depend on which concomitant induction therapy the patient is receiving. Treg may be administered after transplantation, to circumvent the potential negative influence of high doses of corticosteroids used in the first days after transplantation. In addition, it is logical to exclude the use of induction therapy with basiliximab or T-cell depleting therapy, which in theory will inactivate or deplete infused Treg. Another factor which will determine the timing of administration is whether polyclonal or alloantigen-driven Treg are used. The latter will not be available during the first few weeks after transplantation and may therefore not be feasible in deceased donor recipients.

An interesting issue is whether one dose of Treg will be sufficient to induce specific unresponsiveness to donor alloantigen in the longer term. In the ONE study, the intention is to administer one dose of Treg but in subsequent trials, two or more infusions might increase the immune-modulatory effect of Treg therapy, and hence the ability to reduce conventional immunosuppressive treatment. Clearly relevant to this question is the matter of the persistence of the cells after administration. Tracking autologous Treg using conventional techniques such as flow cytometry represents a significant and perhaps insurmountable challenge because the administered cells will share all of the phenotypic markers of their endogenous counterparts and will thus be invisible. In the research setting, cells can be transduced with exogenous reporter genes or labelled with a variety of intracellular dyes, but such approaches are unlikely to be given approval in the context of GMP cell product. Cell tracking by means of gadolinium labelling or deuterium-labelled glucose might provide an answer to the question of how long autologous Treg survive and can be detected after cell infusion [37]. Of note, even without prolonged persistence of infused Treg a sustained immunological shift might occur via 'infectious tolerance' [25,38]. This process refers to the conversion of effector T cells to a regulatory phenotype, meaning that effector T cells that enter the pool of Treg will be converted to Treg, thereby propagating the tolerant state throughout the post-transplant period.

## Practicality issues

The current trials investigating cell-based therapy in transplantation will not only investigate the safety of this therapy, but will also provide a unique opportunity to gain valuable information about practicality issues. For instance, how long before transplantation is it necessary to begin the manufacturing process? This will depend on the type of Treg that will be expanded. In the UK ONE study trial, where the cell product will consist of expanded CD4 + CD25 + nTreg, the total manufacturing time is between 4 and 8 weeks. This time is needed because the expansion process involves CD8+ depletion and CD25+ enrichment followed by 2–3 rounds of stimulation with anti-CD3- and anti-CD28-coated beads in the presence of IL-2 and sirolimus. After final harvest, the cells are cryopreserved whilst formal quality control (QC) assays are performed before product release can be authorized. Whilst these timescales do not present a serious logistical issue in living-donor renal transplantation, cell-based therapy will be problematic in recipients of organs from deceased donors. However, functional and phenotypic data obtained from full GMP engineering runs demonstrate that cryopreserved nTreg remain stable for at least 3 months, corresponding well with that reported elsewhere [39]. If stability for longer periods of cryopreservation can be demonstrated, patients on the transplant waiting list could donate blood for Treg manufacture every 3–6 months, such that therapy with expanded nTreg might be possible for deceased donor transplantation. However, it has to be recognized that this approach would be costly.

Another question relates to the most practical way to obtain a sufficient amount of Treg as a source of the cell product. Current experience with venesection in which 350 ml of blood is taken is promising in the sense that this source population of Treg is sufficient to produce up to $1 \times 10^7$ cells/kg of body weight (unpublished data; Fisher C. and Thirkell S.J.). It is uncertain whether venesection will provide a sufficient yield of Treg if higher concentrations are required. Leukapheresis, shown to be a highly effective source of input nTreg, may be necessary in such cases [40], but may be a problem in patients without vascular access, such as an arteriovenous fistula or a permanent intravenous catheter.

In renal transplantation, the optimal time for procurement of the input cell population is probably predialysis when the haemoglobin level in the patient is relatively high. However, in haemodialysis patients, the timing of blood procurement relative to the dialysis cycle may be extremely important, because some data suggest that dialysis patients have lower numbers of peripheral leucocytes and fewer Treg compared to healthy individuals [41,42]. However, other studies have shown similar numbers [43] or even higher numbers of Treg in dialysis patients as compared to healthy controls [44]. In fact, this study showed that there was no difference in Treg numbers between samples taken pre- and postdialysis [44]. The impact of haemodialysis on leucocyte and Treg numbers might depend on the type of dialysis membrane used [45]. Although the situation is far from clear, it is likely that procuring blood immediately after a dialysis session would lead to an insufficient input population for Treg manufacture suggesting that the optimal time for blood procurement in dialysis patients is after the longest interdialytic interval.

## Expected safety of Treg therapy

The Treg administered in cell therapy trials in solid organ transplantation will be autologous, as opposed to expanded nonautologous Treg used in GvHD trials, suggesting that acute immunological complications would not be expected. Experience with Treg infusion in humans with GvHD, haematopoietic stem cell transplantation, liver transplantation and type 1 diabetes [18,30–33] has shown no serious infusion-related side effects, such as embolic events, anaphylactic responses or cytokine storms. Long-term safety data in humans are sparse, but 1-year follow-up data of 12 young type 1 diabetes patients treated with Treg are promising in the sense that there were no serious adverse events [46]. In this study, one patient developed influenza and another patient developed a mild gastroenteritis of unknown origin 1 day after the cell infusion both being mild and self-limiting. Another patient reported exacerbations of chronic sinusitis that resolved with standard treatment. This suggests that the short-term risks of Treg infusion are likely to be minimal. Experience with Treg therapy in haematopoietic stem cell transplantation shows an increased risk of early viral reactivation as compared to historical controls, mainly driven by an increased incidence of human herpes virus 6 (HHV-6) viraemia [47]. Whether these findings can be extrapolated to solid organ transplantation, in which the risk of clinical disease caused by HHV-6 reactivation is much lower [48], is uncertain. In theory, there may be a small increased risk of malignancy associated with Treg therapy, but this needs to be explored prospectively and compared to the risk with current immunosuppression.

One of the major concerns with Treg therapy is whether the eventual cell product purely consists of Treg or whether it also contains cells with pro-inflammatory characteristics. Using the UK ONE study as an example, isolation of nTreg involves CD8+ depletion and CD25+ enrichment, the latter being a marker not specific for nTreg. One way to reduce the amount of non-Treg in the cell product is the use of sirolimus during the stimulation phase, resulting in preferential enrichment of nTreg [49]. However, due to the lack of unique nTreg markers, the study of human nTreg is challenging, as populations which are isolated based on these markers might not be completely pure. In practice, the number of non-Treg in the eventual cell product will only represent a small proportion of the population already present in the recipient. Based on our own experience so far, the number of alloantigen reactive non-Treg in the cell product will be around 0.01% of the total amount of CD4+ T cells present in an average adult (unpublished data; Hope A.). Although their absolute number is low, these alloantigen reactive non-Treg are probably highly activated and therefore it is difficult to predict what the consequence of this will be. Whilst it is likely that immunosuppressive drugs will control non-Treg in the cell product, it is not clear whether non-Treg that have been driven *in vitro* by bead stimulation + IL-2 will be susceptible to control at normal doses of immunosuppression. To minimize the amount of non-Treg, it is sensible to apply strict release criteria such as: the presence of at least 60-70% CD4 + CD25 + FOXP3+ cells, <10% CD8+ cells, >70% viability, undetectable levels of endotoxin and no growth of bacteria or mycoplasma.

## Potential efficacy of Treg therapy

Performing a trial to investigate the efficacy of Treg therapy will be challenging, particularly when determining the concomitant immunosuppressive treatment. Simply defining a group that will only receive Treg therapy and comparing them with standard treatment will put the participants at an unacceptably high risk of rejection and will certainly not be considered ethical. Therefore, participants who will receive Treg therapy will need at least one immunosuppressive drug such as CNI or mTOR inhibitor monotherapy. The potential detrimental effects of CNIs on Treg might make this regimen suboptimal [50], although animal studies have shown that the combination of low-dose tacrolimus and Treg may lead to long-term graft survival [51]. As mTOR inhibitors do not seem to negatively influence the suppressive function of Treg [52], monotherapy with an mTOR inhibitor might be another option, although mTOR inhibitors cannot be used in the first few weeks post-transplant because of poor wound healing [53]. If the Treg are infused close to the time of transplantation, current induction therapies would be counterintuitive because basilixiamab will deprive the Treg of the essential growth factor IL-2 and alemtuzumab will deplete the cell product as a whole. Interestingly, although induction therapy with ATG depletes most of the leucocyte populations in the peripheral blood [54], it might lead to a relative increase in the number of Treg as opposed to effector T cells. This is exemplified by a study showing that CD25+ T cells are spared from antilymphocyte serum-mediated deletion in mice [55]. Whether *ex vivo* expanded nTreg in humans are unaffected by ATG has not been investigated, but data in splenectomized monkeys suggest prolonged survival of transplanted kidneys when alloantigen-driven Treg are administered during the first 14 days post-transplant after induction therapy with ATG and concomitant sirolimus therapy, compared to treatment with ATG and sirolimus alone [56]. As a consequence of the fact that the efficacy of Treg therapy has to be investigated in conjunction with current immunosuppressive regimens, a long follow-up period and large numbers of patients are needed to show a statistically significant difference in clinical outcomes between the groups with and without Treg therapy.

Finally, the definition of the clinical endpoint will be crucial in determining the power to show efficacy and indi-

rectly, the number of patients needed to be recruited. Two important efficacy measures need to be distinguished. First, rejection episodes, in which one might argue whether protocol biopsies might increase the statistical power to detect changes. Although a protocol biopsy might reveal signs of a lymphocyte infiltrate suspicious of acute rejection, the relevance of these findings is not clear in patients with a stable serum creatinine. The infiltrate seen in a protocol biopsy may consist of Treg and might not necessarily be due to rejection. The second important efficacy measure in cell-based therapy is the reduction in chronic allograft damage, in case cell-based therapy leads to minimization of immunosuppression. Clinical definitions of chronic allograft damage, for example gradual deterioration of graft function, increasing proteinuria and hypertension, are late signs. Therefore, the use of intermediate outcomes such as 'interstitial fibrosis and tubular atrophy (IF/TA)' as a measure of chronic allograft damage in renal transplants might be more appropriate, but will require protocol biopsies [57]. This could be another reason for performing biopsies on all participants in future trials to be able to see subtle changes in efficacy.

## Role of immune monitoring in Treg therapy

Immune monitoring (IM) will play a central part in the evaluation of Treg therapy trials, because it may give a reflection of the degree of immunosuppression and the reactivity of immune cells to donor alloantigen [58]. It may therefore be a useful tool to determine the efficacy of Treg therapy. IM might also be an important step towards individually tailored therapy, in which ideally not only the dosing of Treg but also that of conventional immunosuppression is individualized. The IM assays mentioned below might be useful in clinical practice, although none has been proven to be useful so far.

Immune monitoring assays can be grouped into two main categories; measures of cell therapy safety and measures of the risk of graft rejection. IM assays of the first category which are already performed in clinical practice are the measurement of viral loads of cytomegalovirus, Epstein–Barr virus and BK virus. Another example in this category is more experimental and consists of HLA-DR levels on peripheral blood monocytes as a surrogate marker of innate immunoreactivity, with low levels pointing to immunoparesis. IM assays of the second category are tests that could be used as surrogate markers for transplant outcomes, indicating the risk of rejection. The best-studied IM assay of the second category consists of measuring donor-specific antibodies (DSA), which are increasingly measured in clinical practice. Although the presence of DSA before transplantation is associated with an increased risk of graft rejection, the usefulness of *de novo* DSA as an IM tool after transplantation is still debated, as although it is associated with worse graft outcome [59], there is currently no proven successful treatment for *de novo* DSA. A promising type of IM is gene-expression profiling [60]. Genes that are differentially expressed in tolerant patients might be helpful in identifying patients in which immunosuppression can be safely reduced and might also be helpful in showing the effect of Treg therapy. Other IM assays such as leucocyte subset profiling using flow cytometry [61] have to prove their value before their widespread use in clinical practice.

## Cost-effectiveness of cell therapy

The current cost of manufacturing of cell products might hinder the introduction of cell-based therapy in routine care of transplant patients. Cell products used in ongoing clinical trials are manufactured in fully equipped GMP facilities which results in high manufacturing costs. Manufacturing Treg for 1 patient will cost around £30 000 (approximately $45 000 or €40 000) [62]. In the future, this may fall particularly if semi-automated closed-systems such as the Miltenyi Prodigy device is given regulatory approval for Treg manufacture, but cell manufacture will probably still depend on highly qualified personnel, particularly for product validation and product release. However, if cellular therapy allows successful drug minimization without compromising graft survival its introduction could lead to a reduction in medical expenses, as the annual cost of the current immunosuppression is around £5000 (approximately $7500 or €6750) (www.organdonation.nhs.uk). Further cost reductions could be possible if tapering of immunosuppression leads to a lower incidence of malignancy, infections, diabetes mellitus and hypertension. Whether cell-based therapy with Treg will be cost-effective in the long term has to be tested in future prospective studies. If cellular therapy leads to an increase in transplant survival and allows a reduction in immunosuppression, this will be a tremendous step forward in terms of well-being and quality of life of the patients with end-stage organ disease.

## Conclusion

We are now in an exciting new era with the advent of clinical trials investigating the safety and potential role of Treg therapy in solid organ transplantation. We are about to find out if Treg therapy will live up to the expectations derived from animal studies of the potential to reduce the amount of conventional immunosuppression. If the results of the current clinical trials are promising, we can design future randomized clinical trials to quantify the efficacy of Treg therapy. Showing superiority above the current immunosuppressive regimen might

however prove to be a real challenge as discussed in this review.

## Funding

The authors have received an F7 grant from the European Union to participate in the ONE study: 'A Unified Approach to Evaluating Cellular Immunotherapy in Solid Organ Transplantation'.

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