Published in final edited form as:

Curr Transplant Rep. 2016 December ; 3(4): 265–274. doi:10.1007/s40472-016-0114-9.

Adoptive Cell Therapy with Tregs to Improve Transplant Outcomes: The Promise and the Stumbling Blocks

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Abstract

The contribution of regulatory T cells (Treg) to the induction and maintenance of tolerance is well-recognized in rodents and may contribute to long-term human organ allograft survival. The therapeutic efficacy of adoptively-transferred Treg in promoting tolerance to organ allografts is well-recognized in mouse models. Early phase 1/2 clinical studies of Treg therapy have been conducted in patients with type-1 (autoimmune) diabetes and refractory Crohn's disease, and for inhibition of graft-versus-host disease following bone marrow transplantation with proven safety. The feasibility of adoptive Treg therapy in the clinic is subject to various parameters, including optimal cell source, isolation procedure, expansion, target dose, time of infusion, as well as generation of a GMP-cell product. Several phase 1/2 Treg dose-escalation studies are underway in organ transplantation. Recent evidence suggests that additional factors are critical to ensure Treg safety and efficacy in allograft recipients, including Treg characterization, stability, longevity, trafficking, concomitant immunosuppression, and donor antigen specificity. Accordingly, Treg therapy in the context of organ transplantation may prove more challenging in comparison to other prospective clinical settings of Treg immunotherapy, such as type-1 diabetes.

Keywords

regulatory T cells; cell therapy; transplantation

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Compliance with Ethics Guidelines

Conflict of Interest

Angus Thomson and Mohamed Ezzelarab report a grant from the NOH (AI U01 911297) part of the NIH NHP Transplantation Tolerance Cooperative Study Group and sponsored by the NIAID and NIDDK, during the conduct of this study.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Recent efforts to improve long-term organ allograft survival, while reducing the burden of immunosuppression, have not been particularly successful. The introduction of co-stimulation-blockade (CoSB), while renal-sparing, has resulted in increased incidences of acute rejection [1]. Use of depleting antibody (Ab) as induction therapy at the time of transplant has also not guaranteed safe withdrawal of calcineurin inhibition, even in patients with stable graft function [2, 3]. Furthermore, while strategies to induce donor-specific tolerance using hematopoietic stem cell infusion have yielded promising results, some concerns remain regarding both their safety and generalized application [4, 5]. Thus, there is a need for new therapeutic strategies that may promote tolerance to organ allografts, and that allow the reduction and/or discontinuation of immunosuppressive drugs after transplantation.

Immune cell-based therapies are currently being studied by many centers as an alternative strategy for the promotion of tolerance to organ allografts [6, 7]. These include infusion of donor-derived regulatory macrophages [8, 9], donor- or recipient-derived DCre [10], or myeloid-derived suppressor cells [11]. Regulatory T cells (Treg) are considered particularly promising, based on a large amount of data obtained from both experimental and clinical transplantation studies. However, clinical application of Treg therapy poses numerous challenges [12, 13] and there are many questions that remain to be resolved. In this review, we discuss the recent challenges facing translation of adoptive Treg therapy to the clinic.

Regulatory T cells in transplantation

In rodent organ transplant studies, it has been clearly demonstrated that Treg play a fundamental role in the prevention of allograft rejection and the maintenance of tolerance. However, in clinical transplantation, the role of Treg is less clear. The presence of forkhead box p3 (FoxP3)⁺ Treg in protocol biopsies 6 months after kidney transplant appears to correlate with better graft function at 2 and 3 years. Furthermore, a high FoxP3⁺ Treg/CD3⁺ T cell ratio correlates positively with better graft function, 2 years after transplant [14]. Also, intra-graft FoxP3⁺ Treg with retained FoxP3 demethylation correlate with better outcome in patients with subacute clinical rejection [15]. The same authors have also reported that, in patients that received anti-thymocyte globulin (ATG) and maintenance therapy with rapamycin and mycophenolate mofetil, Treg were elevated both in the peripheral blood and renal graft infiltrates in recipients with low donor-specific Th1 responses (as measured by IFN- γ ELISPOT assay) [16]. The authors suggested that recruitment of Treg to the graft may play a role in its acceptance. However, it has been suggested that FoxP3 expression alone may not be sufficient to distinguish grafts with better outcome [17]. Furthermore, in a selective group of patients receiving conventional immunosuppression (calcineurin inhibition, mycophenolate mofetil and prednisone), acute cellular rejection associated with an increased incidence of CD4⁺FoxP3⁺ T cells in the grafts correlated with less favorable outcome [18]. In a small group of transplant patients that received combined donor bone marrow and kidney allografts from HLA-mismatched donors, long-term donor-specific unresponsiveness was most likely maintained by deletion or anergy, and not by Treg [19]. Considering the variability between these studies in immunosuppressive therapy and timing

of analyses post-transplant, the precise role of Treg in clinical organ transplantation has yet to be determined.

Challenges facing adoptive Treg therapy in organ transplantation

Treg therapy faces certain hurdles, including standardization of manufacturing processes and quality control assessment of the infused product, that are shared with other types of cell therapy. However, some challenges may be more pertinent to Treg than other types of regulatory immune cell therapy, in relation to manufacturing issues, dosage, timing and frequency of cell infusion, specificity, migratory properties and longevity, in addition to the selection of appropriate immunosuppressive agents that do not interfere with or that may promote the desired function of the Treg. Additionally, optimal conditions for Treg safety and efficacy are yet to be determined [20-24, 13]. Furthermore, recent evidence suggests that adoptively-transferred regulatory immune cells may lose their suppressive function under inflammatory conditions in vivo [25].

(i) Characterization of Treg

Traditionally, differential analysis of CD25 (IL-2R α), CD127 (IL-7R α) and Foxp3 expression has been the standard approach to identifying and quantifying naturally-occurring Treg in experimental and clinical studies. Isolation and purification of Treg for functional analysis has been dependent on high expression CD25 and low expression of CD127. However, recent studies suggest that this characterization may be too limited and inadequate to acquire a homogeneous Treg population. Thus, for example, a fraction of human Foxp3⁺ CD4⁺T cells has been shown to lack suppressive function [26].

Various markers have been utilized recently to define Treg based on their origin, function, and migratory capacity. Naïve and memory subsets of Treg have been characterized based on expression of CD45RA (and/or CD45RO) [27]. Furthermore, naturally-occurring and naïve Treg have been shown to express CD31 [27] and Helios [28], markers uniquely expressed by recent thymic emigrants. While the significance of naïve (CD45RA⁺) versus memory (CD45RO⁺) Treg in transplant outcome has yet to be determined, an increased frequency of memory Treg (CD45RA⁻ Foxp3^{hi}) has been observed in kidney transplant patients with operational tolerance, who have been off immunosuppression for at least one year [22]. Meanwhile, activation of Treg has been associated with upregulation of various immune regulatory molecules, such as the adenosine triphosphate (ATP)ase CD39, cytotoxic T lymphocyte antigen 4 (CTLA4), and glucocorticoid-induced tumor necrosis factor receptor (GITR), as well as HLA-DR. Also, recent reports indicate that antigen-specific Treg are more suppressive than polyclonal Treg (see below). Further characterization of allo-antigen specific Treg was achieved by their expression of CD69 and CD71 [29]. Accordingly, Treg can be classified into several subsets based on their phenotype, activation status and origin, adding to the complexity of Treg characterization in vivo.

(ii) Numbers of Treg needed to achieve a therapeutic effect

Under normal, steady-state conditions, Treg numbers remain constant in relation to conventional T cells. In rodent studies of allo-transplantation however, a higher ratio of Treg

to non-regulatory T cells is required to prevent rejection, compared to in vitro studies of Treg function. It has been estimated [30] that Treg should constitute at least 30% of recipient T cells early after transplantation in order to promote tolerance. Being a comparatively rare cell population, <2% of total T cells in the peripheral blood are Treg, where the maximum number of Treg that can be obtained at one time from the peripheral blood of a (healthy) human adult, e.g. by leukapheresis, is around 200×10^6 Treg. Accordingly, ex vivo expansion is essential to obtain sufficient Treg in order to impact the immune response to allografts after transplantation in humans [31].

(iii) Stability and plasticity of Treg

Of particular concern is the stability and potential plasticity of Treg [23, 32-35] that can differentiate into pathogenic effectors under inflammatory conditions [36-38], such as exist in transplant recipients [39, 22, 40]. Recent evidence indicates that under chronic inflammatory conditions [34, 33, 41], including transplantation [35, 42], instability of Foxp3 expression is associated with loss of Treg suppressive function following adoptive transfer.

It has been demonstrated that a minor population of Foxp3⁺ Treg can lose Foxp3 expression under inflammatory conditions and during lymphopenia-induced proliferation, which results in the accumulation of “exFoxp3” T cells [43]. In the periphery, a small population of Foxp3⁺ T cells differentiates into IL-17-producing Foxp3⁻ T cells that accumulate through selective expansion in response to inflammatory cytokine signals. Of note, the authors stated that this observation was not due to “reprogramming” of Treg. Additionally, such IL-17-producing Foxp3⁺ T cells have been observed in inflamed joints and lymph nodes of arthritic mice and in the synovium of human patients with rheumatoid arthritis [41]. Demethylation of the Treg-specific demethylation region (TSDR) at the Foxp3 locus correlates with stability of Foxp3 expression [44], where TSDR is demethylated only in Treg with stable Foxp3 expression, but is fully methylated in CD4⁺ conventional T cells and in in vitro-generated Treg with transient Foxp3 expression.

While it is feasible to grow human Treg to massive numbers for potential therapeutic purposes, it is essential to have a better understanding of the lineage commitment of Treg expanded ex vivo, in order to maintain their stability before and after infusion.

(iv) Concomitant use of immunosuppressive drugs

Choice of immunosuppressive therapy is an important factor in transplant outcome, not simply because it prevents immune responses in allograft recipients, but due to the impact of different immunosuppressive drugs on immune cells, including Treg. Immunosuppressive agents have been classified as being either Treg “friendly” or not, based on their influence on Treg frequency and/or function [45]. For example, patients receiving higher levels of calcineurin inhibitors (CNI) have inferior Treg function and a lower degree of Foxp3 expression and TSDR methylation, compared to patients with lower levels of CNI, or to those receiving rapamycin [46].

(v) Lymphodepletion and Treg

T cell depletion is being used increasingly as induction therapy in clinical organ transplantation, which may affect Treg depending on the lymphodepleting agent used e.g., ATG, Alemtuzumab [anti-CD52mAb] or Daclizumab [anti-IL-2R α mAb]). In rodents [47] and non-human primates (NHP) [48], lymphodepletion is known to induce preferential expansion of Tmem during homeostatic proliferation. Furthermore, lymphopenia-induced T cell proliferation is known to promote inflammatory and autoimmune responses in rodents [49] and humans [50]. Also, lymphopenia-induced proliferation of T cells can result in the generation and accumulation of “exFoxp3” T cells, as mentioned previously [43]. Accordingly, while lymphodepletion initially reduces effector T cells early after transplantation, it may be detrimental to adoptive Treg therapy, either by depletion or associated inflammatory responses.

In rodent models, adoptive transfer of Treg into T cell-depleted [51, 52] or irradiated [53] allograft recipients can prolong graft survival and promote tolerance. When co-transferred with effector T cells into T cell depleted recipient mice, Treg preferentially accumulated in the skin grafts and graft-draining lymph nodes and significantly delayed allograft rejection [51]. Also, ex vivo-expanded donor allo-antigen “enriched” Treg efficiently prevented heart allograft rejection in mice receiving T cell depletion 3 days before and 2 days after transplantation, where Treg were infused on the day of transplant (day 0) [52]. Of relevance, T cell depletion in non-obese diabetic mice with ATG is associated with increased percentage of Treg with a memory phenotype, i.e., CD62L negative [54].

In vitro studies suggest that lymphodepleting agents may promote human Treg. Thus, ATG, but not alemtuzumab or the IL-2 receptor antagonists, induces expansion of CD4⁺CD25⁺ Treg when cultured with human peripheral blood lymphocytes in vitro [55]. Also, coculture of rabbit ATG (but not horse ATG) with human peripheral blood mononuclear cells (PBMC) results in marked expansion of functional Treg [56]. Additional studies indicated that generation of functional human CD4⁺CD25⁺Foxp3⁺ Treg by ATG in vitro requires intact STAT3 (Signal Transducers and Activators of Transcription 3) signaling and the presence of monocytes, and is associated with induction of regulatory CD14⁺CD11c⁺ dendritic cells that are characterized by enhanced IL-10 and decreased IL-12 production [57]. Furthermore, in a randomized clinical trial, kidney allograft recipients who received either ATG or alemtuzumab exhibited increased percentages of CD4⁺CD25⁺ T cells up to 8 months after transplant compared to those who received Daclizumab [58]. However, only those with alemtuzumab induction exhibited a significantly increased Foxp3 mRNA copies in CD3⁺ T cells.

These observations indicate that lymphodepletion early after transplantation may preferentially spare Treg, and may be suitable for adoptive Treg therapy as shown in rodent models. However, the impact of inflammatory responses early after transplantation on adoptive Treg therapy in clinical studies is yet to be determined, particularly with the use of lymphodepletion.

(vi) CD28 costimulation blockade and Treg

The influence of CD28 pathway costimulation blockade using the fusion protein CTLA4Ig on Treg after transplantation remains controversial. Both human effector T cells and Treg may have a similar pattern of CD28 and CTLA4 expression during T cell activation. As CD28 is required for Treg generation and CTLA4 is essential for Treg function, blockade of the interaction of both CD28 and CTLA4 with their ligands CD80 and CD86 on antigen-presenting cells may be detrimental to Treg survival and function, while being essential to prevent effector T cell activation and function. In rodent models, CTLA4Ig accelerated rejection in MHC class II-mismatched heterotopic heart transplantation in which long-term allograft survival is dependent on Treg [59]. In another rodent model of single MHC class II-mismatched skin transplantation, CTLA4Ig prevented Treg-dependent tolerance and restored Th1 alloreactivity and allograft rejection [60]. However, in a recent study, prolonged survival of MHC-mismatched heart allografts was Treg-dependent only when CTLA4Ig was used at low doses, whereas the use of high doses of CTLA4Ig was associated with the loss of the Treg-dependent prolonged graft survival [61]. Accordingly, counterproductive effects of CD28 costimulation blockade on Treg in transplantation models can be avoided by refinement of the dose and timing of its administration. Of relevance, combined use of rabbit ATG, rapamycin and CTLA4Ig (belatacept) was associated with an increased frequency of Treg and their suppressive function in humans [62], one year after transplantation. Collectively, these observations indicate that concomitant and necessary use of immunosuppression with adoptive Treg therapy after transplantation is likely to influence both the fate and function of the infused Treg.

(vii) Longevity and trafficking of Treg

Optimal and appropriate trafficking of Treg is critical to achieve the desired therapeutic effects following infusion. Persistence of Treg after infusion is also essential to maintain long-term therapeutic effects. Both migration and homeostasis of Treg are dependent on the environment in which Treg are infused, as well as on concomitant use of appropriate immunosuppressive drugs. It is not known whether the migration of Treg to secondary lymphoid tissues is essential for their tolerogenic effects in humans, as in mice [63]. In the latter study, Treg migration to mouse allografts was P- and E-selectin ligand and CCR2-, CCR4-, and CCR5-dependent, while Treg migration to draining lymph nodes was CCR2-, CCR5-, and CCR7-dependent. Similarly, prevention of CD62 ligand-dependent migration of allo-antigen specific Treg to lymph nodes abrogated induced tolerance to the allografts [64].

(viii) Treg antigen (allo) specificity

An important limitation of the widely-used immunosuppressive regimens in transplantation is their lack of specificity that results in a “pan”-suppressive effect on the recipient immune system. Allo-antigen (donor)-specific Treg therapy offers the potential to selectively target a specific population of alloreactive effector T cells that are detrimental to the allograft.

In rodents, bona fide antigen-specific Treg are highly suppressive and require T cell receptor stimulation in non-lymphopenic allograft recipients [65]. In turn, antigen-specific Treg are more powerful than polyclonal Treg in promotion of tolerance and indefinite allograft survival [66, 67]. Also, highly suppressive effects of antigen-specific Treg are well-

documented in rodent models of graft-versus-host disease (GVHD) [68]. In a humanized mouse model of chronic rejection [69], human antigen-specific Treg efficiently attenuated the development of arteriosclerosis in transplanted human arteries, induced by allogeneic effector cells. Furthermore, antigen-specific Treg are superior to polyclonal Treg in suppressing immune-mediated injury of human skin allografts in humanized mice [29]. In addition, human allo-reactive (arTreg) have been expanded under good manufacturing practice (GMP)-compliant conditions for generation of clinical-grade human arTreg. These GMP arTreg were also more efficient than polyclonal Treg in prevention of human skin allograft rejection in humanized mice [70]. In a recent report, allo-antigen specific human Treg have been generated using genetic engineering, where Treg expressing chimeric antigen receptors (CARs) can specifically target human allo-antigens in vitro and in vivo in humanized mice. This study provides proof of concept that HLA-specific Treg can be generated for therapeutic purposes [71]. Of note, human CAR T cells (not Treg) infused for the treatment of hematologic malignancies were detected up to several weeks after their infusion [72, 73].

Adoptive Treg therapy in nonhuman primates (NHP)

Immunologic memory, which is more prevalent in humans than in short-lived, inbred, SPF mice, is likely to represent another challenge to adoptive Treg therapy, as human memory T cells are known to be resistant to Treg-mediated suppression [74]. NHP are closer immunologically to humans than to rodents and have high frequencies of alloreactive Tmem [75, 76] that can dominate anti-donor responses following lymphodepletion at the time of transplantation [77-79]. Thus, NHP represent robust pre-clinical models for evaluating promising new therapeutic approaches to achieve transplant tolerance [80].

In a NHP model of kidney transplantation [81], ex vivo-expanded Treg prolonged renal allograft survival in ATG-treated recipients that received numerous infusions (total=14) of expanded, donor alloAg-specific, host spleen-derived CD4⁺CD25⁺ Treg daily (10×10^6 per day). These Treg infusions were started close to ATG administration at the time of transplant into splenectomized recipients, i.e. 24 hours after a 3-day course of ATG (20mg/kg) in combination with low-dose rapamycin for 14 days. This study resulted in prolonged renal allograft survival from 22 to 48.5 days.

Ex vivo-expanded NHP Treg [82-84] have been well-characterized, while endogenous Treg [85], as well as adoptive transfer of ex vivo-expanded Treg or anergic host T cells have been reported to suppress NHP renal allograft rejection [86, 81]. Also, the ability of Treg to suppress alloreactive effector T cell proliferation [87, 83] and their pharmacokinetics in blood and trafficking to host lymphoid tissue following adoptive transfer after lymphodepletion [88, 89] have been studied. In one study by Singh et al, rhesus macaque autologous Treg were eliminated from the peripheral blood in two phases where their half-life was 32.4 ± 11.3 hours in the first phase and 120.4 ± 19.7 hours in the second phase. The rapid elimination of Treg in the first phase was not due to migration to either bone marrow or lymph nodes. Additionally, infused Treg showed a significant decline in CD25 and Foxp3 expression around 6 days after their infusion. In the other study by Zhang et al, autologous versus allogeneic fluorochrome dye-labeled Treg were infused into either normal or ATG

lymphodepleted cynomolgus monkeys. Ex vivo-expanded MHC-mismatched allogeneic Treg did not persist in the peripheral circulation as long as autologous Treg, that were detected in peripheral blood, inguinal LN, mesenteric LN and spleen for >50 days post-infusion [90]. Also, there was a gradual loss of Foxp3 expression following infusion. In both studies, Ki67 expression by infused Treg was not increased, suggesting that the apparent disappearance of the infused dye-labeled Treg from the circulation was not due to proliferation.

Recently, in a NHP (cynomolgus macaque) model of MHC-mismatched heterotopic heart allografts, we infused ex vivo-expanded, polyclonal Treg following ATG-induced lymphodepletion [91]. Our rationale was that, in addition to rapamycin, the therapeutic effect of adoptively-transferred functional Treg would be enhanced in association with depleted endogenous effector T cells [92, 93]. Furthermore, as IL-6 plays a key role in the development of Th17 cells, anti-IL-6R mAb was used to block IL-6 pro-inflammatory effects in an effort to provide stability to the Treg following infusion. Under these conditions, however, multiple Treg infusions resulted in inferior graft function, associated with enhanced circulating memory T cells (**Figure 1**), B cell numbers, and augmented proinflammatory cytokine and anti-donor alloAb levels. The phenotype, TSDR demethylation and suppressive function of infused polyclonal Treg was confirmed in vitro, where the Treg efficiently suppressed proliferative responses of naïve and memory T cells from normal monkeys, as well as of T cells obtained from monkeys acutely rejecting cardiac allografts. The findings indicate that the infused Treg lost their suppressive function in lymphodepleted hosts and may have transformed into pathogenic cells. Despite the use of a Treg-friendly regimen and enhanced Treg to effector ratio by infusion of large numbers of Treg multiple times after transplantation, the host immune environment may perturb their suppressive function

Conclusions

The therapeutic potential of Treg has been validated in rodent models, including humanized mice [94]. These results provide a strong rationale for evaluation of Treg therapy as a therapeutic strategy for auto- or allo-immunity in humans [40]. Adoptive transfer of ex vivo-expanded allogeneic Treg in two patients; one with acute GVHD (who received a total of 3×10^6 Treg/kg) and one with chronic GVHD (who received a total of 100×10^6 Treg/kg), resulted in partial alleviation of symptoms and reduction of immunosuppression, respectively [95]. In patients with HLA-haploidentical stem cell transplantation (HSCT), early infusion of freshly-isolated natural Treg (4 days before transplant) resulted in the prevention of GVHD and promoted immune reconstitution after transplantation [96]. Following allogeneic umbilical cord blood transplantation in humans, infusion of polyclonal ex-vivo expanded Treg – isolated from third-party cord blood – has shown some promise in attenuating acute GVHD in a phase I clinical trial (<https://clinicaltrials.gov>; NCT00602693) [97]. In another study, patients with HSCT were given IL-10-anergized donor T cells (IL-10-DLI) at least one month after transplantation. IL-10 DLI contained regulatory type 1 (Tr1) T cells specific for the host alloantigens, in order to limit donor-vs.-host-reactivity, as well as memory T cells able to respond to pathogens [98]. In this study, four patients (out of twelve)

were alive, in complete remission and without any immunosuppression at least 7 years after transplantation.

Collectively, Treg remain major candidates in tolerogenic adoptive immune cell therapy. Currently, several phase 1/2 Treg clinical trials are underway worldwide in organ transplantation [40, 9, 12] (NCT02088931; NCT02091232; NCT02129881; NCT02188719; NCT02244801). In these clinical trials, different types and target doses of Treg in different organ transplantation settings are being used (**Table 1**). A study published recently by Todo et al [99] reports on the safety and preliminary efficacy of anergic host T cells enriched for Treg in liver donor liver transplantation in which patients were weaned completely off immunosuppression. Transplantation tolerance most likely requires a fine equilibrium between immune regulatory and effector mechanisms. As recent evidence indicates that the inflammatory environment may perturb Treg stability, caution against adoptive Treg therapy under inflammatory conditions, e.g., early post-transplant and following lymphodepletion is warranted.

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Figure 1. Percentages of CD8⁺ effector memory T cells in peripheral blood of lymphodepleted heart allograft recipient cynomolgus monkeys without or with multiple Treg infusions

Heart allograft recipient monkeys received immunosuppression in the form of lymphodepletion with ATG and maintenance with Tacrolimus, rapamycin and IL-6 receptor antagonist, as described [91]. In two recipients, multiple infusions of autologous polyclonal ex vivo expanded Treg were given between days 3 and 31 post-transplant (solid lines). Tmem subsets were identified in peripheral blood based on their differential expression of CD45RA and CD62L. Effector memory (Tem; CD45RA⁺CD62L⁺) CD8⁺ T cells were evaluated sequentially in heart allograft recipients before (Pre-Tx), then 2, 4, 8 and 12 weeks post-transplant. In the control group (with no Treg infusion), percentages of CD8⁺Tem were either modestly increased or remained comparable to baseline. However, CD8⁺Tem were markedly increased above baseline levels at 2 and 4 weeks in the two recipients given multiple Treg infusions.

Table 1

Treg clinical trials in organ transplantation: information obtained from “clinicaltrials.gov”

Trial center (Trial ID)	Organ Tx	Treg					IS
		Type of Treg	Source	Target dose	Frequency	Time of infusion	
UCSF (NCT02188719)	Liver Tx	Donor-Ag alloreactive	Autologous (leukapheresis)	50×10 ⁶ , 200×10 ⁶ , or 800×10 ⁶ (total)	Single infusion	As per protocol	ATG Tacrolimus Everolimus MMF Steroids
UCSF (NCT02711826)	Kidney Tx	Polyclonal or donor-Ag alloreactive	Autologous (leukapheresis)	400 ± 100 × 10 ⁶ (total)	Single infusion	As per protocol	Tacrolimus Everolimus MMF
Germany (NCT02371434) ONE study	Kidney Tx	Polyclonal	Autologous	0.5 × 10 ⁶ , 1 × 10 ⁶ , or 3 × 10 ⁶ /kg	Not available	As per protocol ^{**}	Tacrolimus Everolimus Steroids
UCSF (NCT02244801) ONE study	Kidney Tx	Donor-Ag alloreactive	Autologous (leukapheresis)	300×10 ⁶ , or 900×10 ⁶ (total)	Single infusion	As per protocol ^{**}	NA
MGH (NCT02091232) ONE study	Kidney Tx	Treg-enriched (cocultured with CoSB)	Autologous	NA	Single infusion	Day 7 (+3 days) post Tx	NA
Guy's and St Thomas' NHS Foundation Trust (NCT02129881) ONE study	Kidney Tx	Polyclonal	Autologous	1 – 10 × 10 ⁶ /kg	NA	Day 5 post Tx	Tacrolimus MMF Steroids
UCSF (NCT02474199)	Liver Tx	Donor-Ag alloreactive	Autologous (leukapheresis)	300 – 500 × 10 ⁶ (total)	Single infusion	NA	NA
Hokkaido University (UMIN-000015789) Japan	Liver Tx	Treg-enriched (cocultured with CoSB)	Autologous (leukapheresis)	0.23 – 6.37 × 10 ⁶ /kg	Single infusion	Day 13 post Tx	Cyclophosphamide Tacrolimus MMF Steroids
Guy's and St Thomas' NHS Foundation Trust (NCT02166177) Thrill Study	Liver Tx	Polyclonal	Autologous	low or high dose	Single infusion	NA	ATG Tacrolimus Sirolimus

Abbreviations: CoSB, costimulation blockade; IS, immunosuppression; MMF, mycophenolate mofetil; NA, not available

^{**} Within 60 weeks following renal transplantation biopsy-confirmed acute rejection.