Recent Progress in T_{reg} Biology and Transplant Therapeutics

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Abstract

Purpose of Review Regulatory T cell (T_{reg}) biology continues to evolve at a rapid pace. The role of T_{reg}s in solid organ transplantation offers a unique window into T_{reg} ontogeny and function as well as limitless possibilities for clinical application. Here we review recent significant discoveries and key translational work.

Recent Findings Advances in transplantation deepen understanding of T_{reg} differentiation, expansion, transcription, co-stimulation, and signaling. T cell receptor (TCR) sequencing and single-cell analytics allow unprecedented insight into T_{reg} repertoire diversity and phenotypic heterogeneity. Efforts to replace conventional immunosuppression with T_{reg} adoptive immunotherapy are underway and coalescing around strategies to increase efficiency through development of donor-reactive T_{reg}s.

Summary Adoptive immunotherapy with T_{reg}s is a leading tolerogenic strategy. Early clinical trials suggest that T_{reg} infusion is safe and reports on efficacy will soon follow.

Keywords Regulatory T cells · Tolerance · Adoptive immunotherapy · Kidney transplantation · Liver transplantation

Introduction

Solid organ transplantation provides optimal therapy for patients with end-stage organ failure. The scarcity of available donor organs constitutes a pressing need within the field. Efforts to promote living donation, efficiently capture all eligible deceased donors, utilize marginal donor organs, and rehabilitate injured organs all offer hope. Equally important are efforts to maximize the longevity of each individual organ transplanted working toward the goal of “one organ for life.” In 2017 12.1% of kidney and 9.9% of liver transplants were performed on prior recipients of the same organ [1]. Graft survival is markedly reduced by chronic immune-mediated graft injury and toxic effects of current best available immunosuppressive drugs. There is urgent clinical need to dampen
destructive alloimmunity, enhance regulatory immunity, and replace traditional pharmaceuticals with nontoxic cell-based immunotherapy.

Originally termed “suppressor” T cells, regulatory T cells have been studied for at least 50 years, frequently in the context of transplantation. “Modern” CD4+ Tregs are defined by surface expression of the IL-2 receptor CD25, activity of the Forkhead box P3 (FOXP3) transcription factor, characteristic hypomethylation of genes regulated by FOXP3, and the ability to suppress immune responses in vitro and in vivo. The so-called “natural” Tregs (nTregs) originate in the thymus, while “induced” or “peripheral” Tregs (iTregs or pTregs) are generated through the reprogramming of conventional T cells. Myriad additional subtypes of Tregs are described including Th1-, Th2-, and Th17-like and follicular Tregs (Tfh) [2, 3]. All are major histocompatibility complex (MHC) restricted, and Tregs are known to suppress pro-inflammatory immune responses using both T cell receptor (TCR)-dependent and TCR-independent mechanisms including secretion of anti-inflammatory soluble factors, inhibitory co-stimulation, IL-2 sequestration, antigen-presenting cell (APC) modulation, and direct cytotoxicity [4, 5].

In solid organ transplantation, Tregs are widely viewed as a solution to the challenge of inhibiting destructive donor-reactive immunity while sparing protective host defenses. Epidemiologic studies of patients with Foxp3 mutations clearly establish the importance of Tregs in preventing autoimmune disease [6]. Elucidating the role of Tregs in solid organ transplantation has been more complicated because transplantation is, of itself, a deviation from “natural history.” Limited studies do suggest that patients with Foxp3 mutations have worse transplantation outcomes [7–9] and tolerant kidney transplant recipients have increased indirect pathway regulatory anti-donor T cell responses [10]. There is overwhelming evidence in rodent and primate models that Treg activity can be modulated to prolong allograft survival. Adoptive immunotherapy with Tregs offers the additional potential appeal of replacing nephrotoxic and diabetogenic calcineurin inhibitors with a nontoxic cellular alternative. Here we review major discoveries in the past 3 years that enhance our understanding of Treg function in solid organ transplant and explore ongoing efforts to develop Treg adoptive immunotherapy.

New Molecular Targets and Novel Mechanistic Insights

Despite overwhelming data supporting a role for Tregs in murine allogeneic tolerance, parallel findings supporting causality in human transplant recipients are less common. A recent longitudinal analysis of Treg frequency in living-donor kidney transplant recipients demonstrates that activated alloreactive CD4+CD25hiFOXP3+GARP+ Tregs increase in number approximately 3 months following transplantation [11]. Consistent with the belief that enhanced Treg numbers are favorable, a variety of molecular targets have been manipulated to augment the expansion and survival of Tregs. In a single MHC mismatched skin transplant model, combined administration of donor-specific Tregs and IL-2 synergistically prolonged graft survival and increased numbers of Kd-specific Tregs [12]. Induced expression of the mTOR binding partner DEPTOR in CD4+ regulatory T cells stabilized FOXP3 expression, increased survival and suppressive potency of Tregs, and prolonged survival of fully MHC mismatched murine cardiac allografts [13]. In a murine model of Treg-dependent cardiac allograft survival, overexpression of the complement receptor C5aR2 augmented iTreg induction and prolonged allograft survival [14]. Lastly, in mice and human living-donor kidney transplant recipients, adoptive immunotherapy with human regulatory macrophages enhanced induction of IL-10 producing FOXP3*TIGIT+ iTregs [15].

In related studies, additional cell surface, signaling, and transcriptional targets have been utilized to subtly shift the Treg/Teff balance in favor of allo-acceptance. A prime example is the recent demonstration that the CD45 isoform CD45Rc is not expressed on CD4*FOXP3+ Tregs and transient administration of anti-CD45Rc in a rat cardiac allotransplantation model induced transplant tolerance [16]. Of note, the ability to mount T cell-dependent B cell responses to keyhole limpet hemocyanin were preserved even during anti-CD45Rc administration. At the signaling level, deletion of both the γ and δ variants of PI3 kinase prolonged murine heart allograft survival, but PI3Kδ deletion also reduced Treg survival, suggesting that selective PI3Kγ targeting will be favored in transplant [17]. At the transcriptional level, it is well-known that FOXP3 is essential for expression of lineage-specific target genes in CD4 Tregs, but the roles of other transcription factors are under active investigation. Treg cell-specific conditional knockouts of c-Rel and p65 were used to investigate the role of NF-κB in Treg function [18]. Double conditional knockouts displayed a severe autoimmune Scurfy-like [19] phenotype, and subsequent RNA-seq experiments confirmed that NF-κB helps maintain the identity and function of mature Tregs. Both the NF-κB and IRF transcriptional pathways are potential targets in transplantation, and recently deletion of transcription factor IRF4 in CD4+ T cells caused upregulation of the Treg-associated markers Helios and PD-1, resulting in disordered immunity and transplant acceptance [20].

Co-stimulatory blockade continues to evolve as a strategy in transplantation. With > 10 years of clinical data now available, belatacept is now familiar in renal transplantation, and αCD40/CD40L therapy, originally plagued by problems with thromboembolism, is resurfacing [21]. Selectively disrupting checkpoints while preserving Treg function is essential, and to that end Wood and colleagues compared CTLA4-Ig with selective antibody blockade of CD28 in a humanized murine
skin transplant model [22•]. Anti-CD28 demonstrated superiority, likely in part by leaving CD80/86 available to engage CTLA4 present on Tregs.

Perhaps most unexpected are recent reports on donor-derived Tregs. Pettigrew et al. demonstrate persistence of donor-derived nTregs in human lung transplant recipients [23•]. Pursuing this observation in a murine cardiac transplant model, they demonstrate that deletion of donor CD4 nTregs before organ recovery accelerated allograft rejection and show that donor-derived nTregs were more efficacious than recipient-derived nTregs in restoring allograft survival. In similar experiments, Sachs and colleagues report that long-term tolerant swine kidney grafts confer infectious tolerance when re-transplanted implying the presence of a strong intra-graft regulatory element [24]. In recognition of the importance of Treg locale, culture conditions favoring CXCR3, α4β7 integrin, and CCR9 were used to tailor the homing capacity of Tregs to tissue sites of interest [25].

Because Tregs are able to suppress through both TCR-dependent and TCR-independent mechanisms, the transplant community has sought to utilize both polyclonal and donor antigen reactive Tregs in a therapeutic capacity. Consensus opinion now seems to accept that efficacy will be greatest when donor-reactive Tregs are utilized but larger questions remain concerning the true size of the human alloresponse and the diversity of the Treg TCR repertoire compared with that of conventional T cells. Advances in next-generation sequencing and big data analysis have enabled recent breakthroughs with relevance across disciplines. Shen and colleagues revisited the age-old question of alloreactive frequency using modern approaches and found 0.5–6% of the circulating TCR repertoire reactive to just two different allogeneic stimulators, reproducing the antiquated conventional estimate of 1–10% with remarkable accuracy [26•]. The TCR repertoire of Tregs is as diverse, if not more so, than the repertoire on naïve CD4+ T cells [27], and thus, we can infer broad clonal diversity within populations of donor-reactive Tregs. Lastly, Benoist and colleagues used single-cell RNA-Seq to profile thousands of mouse and human Tregs and found that while extensive phenotypic diversity exists, the main features of Treg heterogeneity are similar in mice and humans [28], providing validation to the relevance of murine studies.

Active Strategies for Translation

Efforts to convert our evolving understanding of Tregs in transplantation to safe human therapies primarily involve Treg adoptive immunotherapy (Fig. 1). This includes bulk transfer of polyclonal Tregs, transfer of “tailored” donor-reactive populations, combined kidney bone marrow transplantation, and adoptive transfer of T cells engineered to express TCRs, antibodies, or protein antigens that direct Treg function in an antigen-specific manner.

Adoptive Immunotherapy with Polyclonal and Donor-Reactive Tregs

Recipient peripheral blood is the primary source of Tregs for ex vivo expansion and subsequent adoptive immunotherapy; however, reports utilizing umbilical cord blood-derived Tregs are emerging [29, 30], and West and colleagues report the intriguing prospect of using human thymus routinely removed during pediatric cardiothoracic surgery as a source of nTregs [31]. Numerous manuscripts addressing the technical aspects of clinical Treg manufacture including cryopreservation [32] and automation [33] have appeared. Major outstanding issues are optimization of culture conditions for ex vivo Treg expansion, strategies for directing donor reactivity, and compatibility of various Treg products with conventional immunosuppression required in clinical trials.

Typical Treg expansion strategies involve magnetic bead or flow cytometric enrichment of a CD4+CD25+ cell population (sometimes with additional selection based upon CD127 or CD45RA), which is then expanded several thousand-fold in culture bags or bioreactors containing serum-enhanced media, IL-2, and TCR and co-stimulatory signals most often provided via αCD3/αCD28 antibodies. Media is further modified with rapamycin, cytokines, the vitamin A metabolite all-trans retinoic acid, amino acids, and short chain fatty acids to enhance purity and tailor Treg functionality. In comparing CD45RA positive and negative CD4+CD127–/loCD25+ cells cultured in the presence of tacrolimus, Wood and colleagues found that although CD45RA– Tregs have greater suppressive capacity post-expansion, they do not retain a stable TSDR demethylated phenotype raising concerns that these cells might become pathogenic in transplant recipients [34]. Marti and colleagues compared ex vivo Treg expansion with rapamycin and everolimus and found, despite differing kinetics, equivalence in the final Treg product supporting consistent in and ex vivo use of the clinically favored drug everolimus [35]. Early investigation into the mTOR inhibitory activity of azithromycin shows no clear advantage over rapamycin [36], and stimulation of naïve CD4+ T cells in media containing low tryptophan and kynurenines has been shown to foster development of iTregs [37]. Markmann’s group reports successful Treg generation from peripheral blood of uremic pre-transplant candidates using ex vivo MLR and belatacept co-stimulatory blockade [38].

Efforts to promote anti-donor reactivity add considerable complexity to Treg expansion protocols. While unmodified peripheral blood has been successfully used as a source of donor antigen-presenting cells [38–40], donor B cells activated with CD40L-expressing feeder cells are typically used to
capitalize on the relative abundance (vs. dendritic cells) and potent stimulatory capacity of B cells. To bypass concerns that CD40L+ feeder cells contaminating T<sub>reg</sub> preparations would cause indiscriminant activation of alloreactive effector T cells, Leventhal and colleagues tested B cell activation and expansion using soluble 4-trimer CD40 ligand and successfully converted naïve CD4 T cells to demethylated T<sub>regs</sub> with a constricted donor-reactive TCR repertoire [41].

Adoptive immunotherapy (AI) and conventional immunosuppression will be co-administered, at least until non-inferiority of T<sub>reg</sub> AI is proven, and thus there is great interest in understanding how conventional immunosuppression affects endogenous T<sub>reg</sub> numbers and synergizes with adoptive T<sub>reg</sub> therapy. Amirzargar et al. compared T<sub>reg</sub> number and phenotype in 24 renal transplant recipients treated with either tacrolimus/mycophenolate/prednisone or tacrolimus/sirolimus/prednisone therapy and proved that the latter was favorable in augmenting T<sub>reg</sub> numbers and reducing RORγt expression associated with T<sub>reg</sub> conversion to a pro-inflammatory Th17 phenotype [42]. Lombardi and colleagues show that rapamycin-treated ex vivo-expanded human T<sub>regs</sub> maintain a stable T<sub>reg</sub> phenotype in the presence of tacrolimus, mycophenolate, and methylprednisolone; however, tacrolimus altered chemokine receptor expression and reduced IL-10 production [43]. All three agents

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**Fig. 1** Schematic illustrating four distinct approaches to T<sub>reg</sub> immunotherapy. (1) Infusion of polyclonal T<sub>regs</sub>, (2) infusion of T<sub>regs</sub> with known anti-donor reactivity, (3) combined kidney and bone marrow transplantation with mixed chimerism, and (4) infusion of T cells bearing transgenic receptors (T cell receptors, chimeric antigen receptors, B cell antigen receptors) engineered with anti-donor reactivity. (Figure created using Biorender.com)
reduced viability, function, and proliferative capacity relative to rapamycin in a humanized mouse model.

**Published Clinical Trials**

Of the four published clinical trials utilizing Treg adoptive immunotherapy, two involve liver transplant which is widely accepted as a less immunogenic transplant than kidney. Okumura and colleagues co-cultured recipient lymphocytes and irradiated donor lymphocytes in the presence of αCD80/86 monoclonal antibodies which generated Tregs with donor reactivity in mixed lymphocyte reactions [44]. Ten splenectomized living-donor liver transplant recipients received this Treg product 13 days after transplantation and, notably, after administration of 40 mg/kg of cyclophosphamide on postoperative day 5. Immunosuppression was weaned between months 6 and 18 post-transplant, and seven of ten patients successfully discontinued immunosuppression. The trial was halted because three patients with primary biliary cirrhosis or primary sclerosing cholangitis as the cause of their liver failure developed treatable acute cellular rejection. Lombardi and colleagues add to this with their recently published open-label, dose escalation, phase I clinical trial of autologous polyclonal Treg therapy [45]. Polyclonal Tregs were grown from recipient peripheral blood in the presence of αCD3/CD28, IL-2, and rapamycin, and 0.5–1 million Tregs/kg or 3–4.5 million Tregs/kg were administered to nine cadaveric liver transplant recipients at least 3 months after transplantation. No attempts were made to wean immunosuppression. One patient experienced an infusion-related cytokine storm. Infusion transiently increased the pool of circulating Tregs, and the study was not powered to address therapeutic efficacy.

Two differing approaches to Treg adoptive immunotherapy in renal transplantation have been published to date. Vincenti and colleagues tested the safety and feasibility of autologous polyclonal Treg therapy in patients with subclinical inflammation on 6-month surveillance biopsies [46]. Three renal transplant recipients with 6-month biopsies demonstrating 5–25% inflammation (Banff i0 or i1) and no evidence of rejection (Banff i &lt; 2, t &lt; 2) received 320 × 10^6 autologous CD4^+CD127^lowCD25^+ polyclonal Tregs isolated from peripheral blood via FACS sorting and expanded in the presence of αCD3/αCD28, IL-2, TGFβ, and sirolimus [47]. Treg infusion was safe. Conventional immunosuppression with sirolimus and mycophenolate was maintained. In 2 years of follow-up, one patient developed subclinical rejection, and two patients developed de novo DSA.

Overall, the published clinical trials to date suggest that both polyclonal and donor-reactive Treg products can be safely manufactured and administered. Feared complications including over-immunosuppression, infection, malignancy, and conversion of infused Tregs to a destructive alloreactive phenotype have not been observed. The efficacy of Treg adoptive immunotherapy and optimal approach in each clinical scenario are open questions, and a large number of additional clinical trials are currently in progress (Table 1).

**Combined Kidney Bone Marrow Transplantation**

Bone marrow transplantation is perhaps the most extreme form of adoptive immunotherapy, and combined kidney bone marrow transplantation (CKBMT) with transient mixed chimerism has been shown to induce long-term tolerance in human recipients [48–50]. The long-term success of this strategy relies upon deletion of donor-reactive clones [51]. Investigation into the complex mechanisms allowing for clonal deletion allows opportunity to study Tregs with proven clinical efficacy. In the nonhuman primate CKBMT model, CD4^+FOXP3^+ T cells proliferating in response to donor antigens in the CFSE mixed lymphocyte reaction were shown to be iTregs converted from conventional T cells [52]. In human CKBMT recipients, both new thymic emigration and lymphopenia-driven proliferation were shown to account for the marked early enrichment of CD4^+CD25^highCD127^lowFoxp3^+ cells in peripheral blood [53]. Sykes and colleagues have introduced the novel technique of using activated B cells to expand donor-reactive Tregs pre-transplant [54]. Expansion facilitated deep sequencing and allowed for clonal tracking which ultimately demonstrated that preexisting donor-reactive Tregs were expanded at 6 months post-transplant in tolerant human CKBMT recipients and failed to expand in a non-tolerant recipient. Kawai’s group used a series of allo-graft biopsies from nonhuman primate CKBMT recipients to establish an mRNA signature of tolerance that included a large number of Treg-associated transcripts including FOXP3, IL10, TGFβ, and GATA3 [55]. Further, they have demonstrated that their combined CKBMT approach does not induce tolerance to islet allografts in nonhuman primates [56]. Lastly, they have recently demonstrated that the addition of αCD40 monoclonal antibody to their mixed chimerism approach abrogates tolerance induction and they speculate that the mechanism involves a defect in antigen presentation to regulatory cells [57].
### Table 1  Summary of clinical trials involving T\textsubscript{reg} adoptive immunotherapy in solid organ transplantation listed on clinicaltrials.gov

| Study                                                                 | Indication                                                                 | Phase | Study ID          | Product                                                                 | Status                      |
|----------------------------------------------------------------------|---------------------------------------------------------------------------|-------|-------------------|-------------------------------------------------------------------------|-----------------------------|
| Infusion of T-Regulatory Cells in Kidney Transplant Recipients (The ONE Study) | Kidney failure, kidney transplant                                         | I     | NCT02091232       | Polyclonal T\textsubscript{regs}                                        | Active, not recruiting     |
| A Pilot Study Using Autologous Regulatory T Cell Infusion Zortress (Everolimus) in Renal Transplant Recipients | End-stage renal disease/kidney transplant                                 | NA    | NCT03284242       | Polyclonal T\textsubscript{regs}                                        | Recruiting                 |
| Donor Alloantigen Reactive Tregs (darT\textsubscript{reg}) for Calcineurin Inhibitor (CNI) Reduction (ARTEMIS) | Liver transplant recipient/living-donor                                   | I/II  | NCT02474199       | Dar T\textsubscript{regs}                                               | Active, not recruiting     |
| Treg Therapy in Subclinical Inflammation in Kidney Transplantation (TASK) | Kidney transplant/adult living-donor kidney transplant recipients/renal transplant/living kidney donor | I/II  | NCT02711826       | Polyclonal T\textsubscript{reg}/everolimus/tacrolimus/mycophenolate mofetil/mycophenolic acid | Recruiting                 |
| Liver Transplantation With Tregs at MGH (LIITMUS-MGH)                | Liver transplant                                                           | I/II  | NCT03577431       | Dar T\textsubscript{reg}/cyclophosphamide/mesna/everolimus             | Recruiting                 |
| TLI, ATG & Hematopoietic Stem Cell Transplantation and Recipient T\textsubscript{reg} Therapy in Living Donor Kidney Transplantation | Living-donor kidney transplanation                                         | I     | NCT03943238       | Donor hematopoietic stem cells/recipient T\textsubscript{reg}          | Not yet recruiting         |
| Treatment of Children With Kidney Transplants by Injection of CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} T Cells to Prevent Organ Rejection | Kidney failure/end-stage renal disease                                     | I/II  | NCT01446484       | Polyclonal T\textsubscript{reg}/alemtuzumab/mycophenolate mofetil/sirolimus/tacrolimus/cyclosporine/everolimus | Recruiting                 |
| The ONE Study nT\textsubscript{reg} Trial (ONEnTreg13)                | Immunosuppressive treatment of living-donor renal transplantation          | I/II  | NCT02371434       | Polyclonal T\textsubscript{reg}                                        | Recruiting                 |
| T\textsubscript{reg} Adoptive Therapy for Subclinical Inflammation in Kidney Transplantation (TASK) | Late complication from kidney transplant                                  | I     | NCT02088931       | Polyclonal T\textsubscript{reg}                                        | Completed                  |
| The ONE Study UK T\textsubscript{reg} Trial                           | End-stage renal failure                                                   | I/II  | NCT02129881       | Polyclonal T\textsubscript{reg}                                        | Completed                  |
| Donor-Alloantigen-Reactive Regulatory T Cell (darT\textsubscript{reg}) Therapy in Renal Transplantation (The ONE Study) (DART) | Kidney disease                                                            | I     | NCT02244801       | Dar T\textsubscript{reg}                                               | Completed                  |
| Trial of Adoptive Immunotherapy With TRACT to Prevent Rejection in Living Donor Kidney Transplant Recipients (TRACT) | End-stage renal disease                                                   | I     | NCT02145325       | Polyclonal T\textsubscript{reg}                                        | Completed                  |
| Safety and Efficacy Study of Regulatory T Cell Therapy in Liver Transplant Patients (ThRIL) | End-stage liver disease                                                  | I/II  | NCT02166177       | Polyclonal T\textsubscript{reg}                                        | Completed                  |


**TCRs, CARs, and BARs**

“Manufacturing” recipient-derived donor-reactive Tregs poses significant challenges, particularly in the setting of cadaveric transplantation where the time interval between donor selection and transplantation can be short. Redirecting the specificity of Tregs via gene transfer of donor-reactive TCRs, antibody-based fusion proteins specific for allo-MHC (CAR), or the antigenic targets of B cells (BAR) are promising alternative strategies under development. Attempting to capitalize on the prevalence of HLA-A2 in many donor populations, two groups report creation of HLA-A2-specific Treg CARs that display potent suppressive capacity in vitro and the ability to suppress GVHD and skin transplant rejection in humanized mouse models [58, 59]. Boardman et al. have begun to explore mechanisms of Treg CAR function and utilize regulatory CARs with mutated intracellular signaling domains to show convincingly that signaling through the CAR is essential for suppressive function [60]. Meyer and colleagues experiment with transient transfection and offer an elegant platform in which a single CAR can accommodate multiple specificities [61]. By coupling CD28 and CD3ζ signaling domains with an anti-FITC scFv, FITC-conjugated antibodies of various specificities can be added to modulate this single “platform.” Using FITC-conjugated anti-donor HLA class I monoclonal antibodies, they facilitate the homing of Tregs to pancreatic islets placed under the kidney capsule. Surprisingly, these “mAbCAR” Tregs remain localized near the islets long after expression of the transgene is lost suggesting that transient CAR expression “parades” polyclonal Tregs through the effector site with retention and/or proliferation of donor-reactive clones. Concerns surrounding CAR therapy involve insertional oncogenesis, graft versus host disease, and off-target expression of effector function, but to date these have not been problematic in animal models. BAR therapy, intended to be useful in recruiting regulatory cells to germinal centers to prevent anti-donor antibody formation, is also under active development [62].

**Summary and Future Challenges**

Alloimmune responses involve a balance between effector and regulatory T cell activity. Recent work highlighted here enhances understanding of Treg origin, development, and effector function. Enhancing Treg activity in solid organ transplantation offers the hope of reducing or eliminating current noxious immunosuppressive drugs. Although donor-reactive Tregs display broad clonal and phenotypic diversity, strategies to harness donor-reactive Tregs for adoptive immunotherapy are plausible, and early clinical trials suggest that the approach is safe. Highly anticipated results from a number of ongoing trials are likely to enable a new era of biologic immunotherapy. Critical challenges include (1) the need for strategies to create donor-reactive Tregs that can be administered at the time of transplantation within the logistic constraints imposed by both living-donor and cadaveric-donor transplantation, (2) identification of ideal Treg phenotypes and optimization of ex vivo expansion conditions that preserve these phenotypes, and (3) the need to understand compatibility with existing immunosuppressive regimens to ensure that adoptive immunotherapy trials remain both safe and rational.

**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

**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.

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