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Regulatory T cell therapy in solid organ transplantation: clinical applications and laboratory monitoring strategies

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Regulatory T cells (Tregs) play a fundamental role in maintaining immunological homeostasis and sustaining immune tolerance. In solid organ transplantation, Tregs have emerged as a promising strategy of cellular immunotherapy capable of reducing allograft rejection and mitigating long-term complications. Early-phase clinical studies have established the safety and feasibility of adoptive Treg therapy. Building on these pivotal findings, Treg therapy has created a solid foundation for translational application. However, successful clinical implementation requires a deeper understanding of Treg biology, clarification of their mechanisms of action, and the development of reliable strategies for *in vivo* monitoring. This review provides a detailed overview of Treg mechanisms, ongoing clinical trials, and methodological approaches for evaluating their phenotype and function through cell-based, protein-based, and gene-based assays. In addition, it highlights key considerations for optimizing therapeutic efficacy and ensuring safety, with the ultimate aim of advancing Treg therapy toward routine clinical use in solid organ transplantation.

Keywords: Transplantation; Regulatory T cell; Immunotherapy; Immunologic monitoring

INTRODUCTION

Regulatory T cells (Tregs), identified primarily by the expression of CD4, CD25, and the transcription factor FOXP3, constitute approximately 1% to 10% of the circulating CD4+ T cell population [1–3]. These cells play a central role in maintaining immune homeostasis and tolerance by suppressing the activation and proliferation of effector T cells, dendritic cells, and other immune components [4]. Their immunosuppressive activity is mediated through several mechanisms, including metabolic disruption, modulation of antigen-presenting cell (APC) function, secretion of immunosuppressive cytokines, and direct cytolysis of target cells [5].

In solid organ transplantation (SOT), conventional immunosuppressive regimens have successfully reduced the incidence of acute rejection. Nevertheless, long-term complications, including opportunistic infections, malignancies, and chronic allograft dysfunction, remain significant challenges [6,7]. In contrast, Treg therapy provides a targeted strategy to promote donor-specific tolerance while minimizing complications and preserving overall immune competence [8]. Preclinical studies employing *ex vivo*-expanded Tregs or donor antigen-specific Tregs (darTregs) have shown prolonged graft survival with reductions in both rejection and graft-versus-host disease [9–11]. These results support early-phase clinical trials aimed at investigating the immunological effects, safety,

HIGHLIGHTS

- Regulatory T cells (Tregs) play a central role in immune tolerance after solid organ transplantation.
- Early clinical trials have demonstrated the safety and potential of Treg therapy.
- Laboratory assays enable phenotypic and functional monitoring of Tregs.
- Integrated strategies are needed to optimize Treg therapy and its monitoring.

and feasibility of Treg therapy in SOT.

Despite these advances, widespread clinical translation of Treg therapy faces major challenges, particularly the absence of standardized and robust assays capable of reliably monitoring phenotypic stability, suppressive function, persistence, and *in vivo* stability [12,13]. Conventional assays, such as enumeration of total Tregs based on surface markers and viability measurements, provide only limited insight into suppressive capacity or antigen specificity [14,15]. Furthermore, these basic assessments may not reliably correlate with clinical outcomes or immune tolerance status [16,17].

Recent technological developments, including functional suppression assays, cytokine profiling, and epigenetic or single-cell analyses, provide opportunities for more precise immunological monitoring [18,19]. Importantly, these approaches highlight the potential for precision medicine in transplantation, enabling a shift from uniform immunosuppressive regimens to individualized, cell-based therapeutic strategies.

This review offers a comprehensive overview of Treg mechanisms of action, the current progress of clinical trials, and available methodologies for their evaluation. Special attention is given to emerging laboratory technologies that facilitate the monitoring of Treg phenotype, function, and stability using cell-based, protein-based, and gene-based platforms. Moreover, the review discusses the present limitations of Treg therapy and outlines future directions to enhance its safety, efficacy, and applicability in SOT.

REGULATORY T CELLS IN TRANSPLANTATION: MECHANISMS AND CLINICAL TRANSLATION

Tregs play a pivotal role in maintaining immune tolerance through diverse mechanisms, including metabolic disruption, contact-dependent inhibition, cytokine production, and induction of cell death. Building on these fundamental immunoregulatory functions, Treg therapy has emerged as a promising strategy to promote immune tolerance in transplantation. Recent advances in the isolation, expansion, and engineering of Tregs have enabled the development of therapeutic approaches with improved specificity and stability. Ongoing clinical trials are actively assessing the safety, efficacy, and optimal protocols for Treg therapy across different clinical applications.

Action of Regulatory T Cells

Tregs employ multiple distinct immunosuppressive mechanisms to prevent alloimmune responses by suppressing activated effector immune cells (Fig. 1). One key mechanism is the metabolic disruption of essential immune resources [5,20]. Tregs constitutively express high levels of the high-affinity interleukin (IL)-2 receptor alpha chain (CD25), which allows them to efficiently sequester IL-2 [21,22]. This limits IL-2 availability for effector T cells and natural killer cells, ultimately inducing apoptosis of activated effector cells [23,24]. In addition, Tregs stimulate APCs to produce indoleamine 2,3-dioxygenase (IDO) [25]. IDO and other enzymes that catabolize amino acids such as tryptophan and arginine contribute to a metabolic environment unfavorable for effector T cell proliferation [25,26]. A second major mechanism is direct contact-dependent inhibition of APCs [5,20]. Tregs express inhibitory receptors, including cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and lymphocyte activation gene 3 (LAG-3), which interact with CD80/86 and major histocompatibility complex II molecules on APCs, respectively [27,28]. These interactions trigger trans-endocytosis, reducing APC costimulatory molecule expression and impairing effector T cell activation [29,30]. A third mechanism involves secretion of immunosuppressive cytokines, such as IL-10, transforming growth factor beta (TGF-β), and IL-35 [31,32]. These cytokines inhibit interferon-γ–producing T helper 1 (Th1) cells and IL-17–producing Th17 cells, while promoting the expansion and stabilization of other regulatory cell subsets [33,34]. A final major mechanism is direct cytotoxicity against effector T cells and APCs through granzyme-dependent apoptotic path-

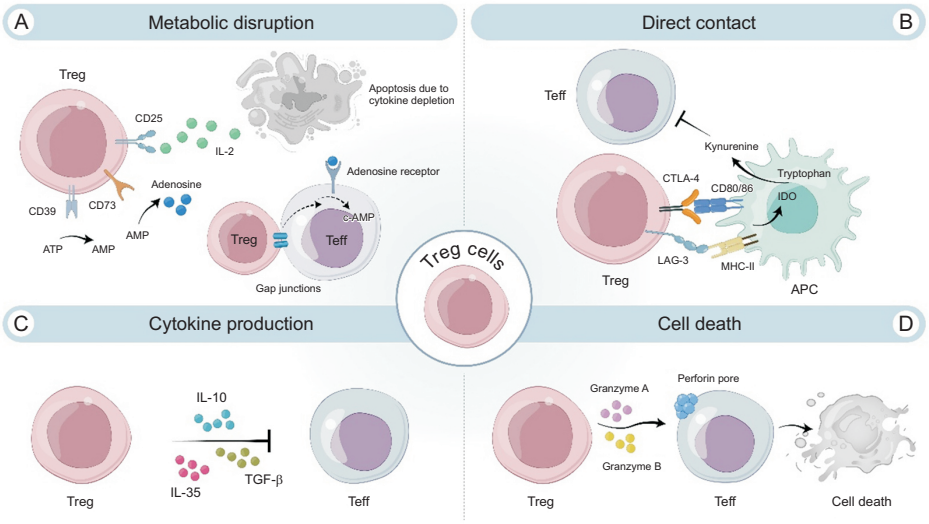


Fig. 1. Mechanisms of immune tolerance mediated by regulatory T cells (Tregs) in solid organ transplantation. Tregs primarily employ four suppressive mechanisms to maintain immune tolerance and prevent allograft rejection. (A) Metabolic disruption: Tregs express high-affinity interleukin (IL)-2 receptors (CD25), enabling them to competitively consume IL-2 and deprive effector T cells (Teffs), leading to their apoptosis. CD39 and CD73 expressed on Tregs convert extracellular ATP into immunosuppressive adenosine, which suppresses effector T cell activity via adenosine receptors. Tregs also transfer cyclic adenosine monophosphate (c-AMP) to Teffs through gap junctions, further impairing their activation. (B) Direct contact–dependent inhibition: Tregs suppress antigen-presenting cells (APCs) by expressing cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and lymphocyte activation gene 3 (LAG-3), which bind CD80/CD86 and major histocompatibility complex (MHC)-II, respectively. These interactions downregulate costimulatory signaling. Additionally, CTLA-4 engagement induces indoleamine 2,3-dioxygenase (IDO) expression in APCs, leading to tryptophan depletion and kynurenine generation, both of which inhibit effector T cell proliferation. (C) Suppressive cytokine production: Tregs secrete immunoregulatory cytokines, including IL-10, transforming growth factor beta (TGF-β), and IL-35, which inhibit activation and proliferation of Teffs and other immune cells. (D) Induction of cell death: Tregs release granzyme A, granzyme B, and perforin, inducing apoptosis in Teffs.

ways [35–37]. Other mechanisms, such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/death receptor DR5 interactions and galectin-mediated cytolysis, have also been described [38,39].

Together, this diverse repertoire of suppressive mechanisms enables Tregs to dynamically regulate immune responses. In the setting of SOT, Tregs act to balance prevention of graft rejection with preservation of protective immunity [40,41].

Regulatory T Cell Therapy: Evolving Strategies in Transplantation

Because of their central role in regulating alloimmune responses, Tregs have emerged as promising therapeutic candidates for donor-specific immunomodulation in SOT [41]. To translate this potential into clinical application, multiple strategies have been developed to expand Tregs and enhance their antigen specificity. These strategies can be broadly categorized into three types: polyclonal Tregs, darTregs, and chimeric antigen receptor (CAR)-Tregs (Table 1).

Polyclonal Tregs can be expanded either *ex vivo* or *in vivo* without specific antigen stimulation [42]. *Ex vivo* expansion typically employs nonspecific stimuli, such as anti-CD3/CD28 antibodies with IL-2, under controlled laboratory conditions [43–45]. Polyclonal Tregs exhibit broad antigen reactivity and are relatively simple to manufacture; however, their lack of antigen specificity may necessitate infusion of large cell numbers to achieve therapeutic efficacy [46]. In contrast, *in vivo* expansion strategies rely on administration of low-dose IL-2 to selectively promote endogenous Treg proliferation in recipients [47–49]. In this context, the Transplantation Without Overimmunosuppression (TWO) study evaluated autologous polyclonal Treg therapy in living donor kidney transplantation. This trial showed that polyclonal Treg therapy enabled successful minimization of immunosuppression with low-dose tacrolimus monotherapy, leading to fewer complications and stable long-term graft function [50].

To improve specificity, darTregs are induced by coculturing *ex vivo*-expanded Tregs with donor-derived alloantigens or donor cells [51]. Clinical protocols for darTreg expansion have employed CD40L-stimulated B cells or

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Table 1. Comparative overview of Treg-based immunotherapies in transplantation

Feature	Polyclonal Tregs	darTregs	CAR-Tregs
Mechanism of action	• *Ex vivo*-expanded Tregs that react to a wide range of antigens	• Selected and expanded Tregs that specifically recognize donor-alloantigens via native TCRs	• Genetically engineered Tregs expressing CARs for enhanced antigen specificity
Antigen specificity	• Low • Broad and nonspecific	• High • Targeted to donor antigen	• Very high • Engineered to recognize a defined antigen (e.g., HLA-A2, tissue-specific antigens)
Persistence (*in vivo*)	• Limited • Typically detectable for days to weeks after infusion; may not persist long-term	• Intermediate • Shown to be detectable in tissues for a limited period, may wane in weeks	• Variable • Shown to persist in animal models for weeks; human data is limited but may offer improved persistence
Clinical trial outcomes	• Shown to be safe in early-phase trials (e.g., kidney, liver transplant). • Some success in reducing immunosuppression	• Early trials (e.g., the ONE Study) suggest potential in promoting donor-specific tolerance • Ongoing studies underway	• Preclinical models show strong efficacy in suppressing rejection • Human trials are in early stages
Advantages	• Easier to manufacture; extensively studied; broadly applicable	• Greater efficacy with fewer cells; targeted suppression	• Highly specific; potential for durable and localized tolerance
Limitations	• Nonspecific; may require repeated dosing; risk of off-target effects	• Technically challenging; requires donor-recipient matching and *ex vivo* expansion	• Engineering complexity; unknown long-term safety and efficacy; regulatory hurdles

Treg, regulatory T cell; darTreg, donor-specific regulatory T cell; CAR, chimeric antigen receptor; TCR, T cell receptor; HLA, human leukocyte antigen.

low-dose IL-2 combined with rapamycin [52,53]. These approaches demonstrated enhanced suppressive potency and antigen specificity in humanized mouse models. The ONE Study consortium further evaluated the safety and feasibility of darTreg administration in kidney transplantation [54]. By selectively recognizing and suppressing donor-specific immune responses, darTregs provide the strongest clinical evidence supporting targeted immunotherapy [55]. However, both polyclonal and darTreg therapies face limitations in yield, as Tregs represent only 3% to 17% of the final cell product. Thus, optimal dosing requirements and the degree of antigen specificity needed for therapeutic success remain unresolved [56,57].

CAR-Tregs represent the most advanced approach, employing genetic engineering to confer precise donor specificity [58]. These cells are engineered to express synthetic receptors that recognize donor human leukocyte antigen (HLA) molecules on graft tissues [59,60]. CAR-Tregs offer distinct advantages, including uniform antigen specificity, lower therapeutic cell requirements, improved scalability, and preservation of natural T cell receptor (TCR)-mediated reactivity.

Collectively, these evolving strategies signify a paradigm shift from broad immunosuppression to targeted immune regulation. Progress in Treg therapy underscores the urgent need for standardized laboratory monitoring methods to evaluate Treg stability, function, and *in vivo* persistence, which are key requirements for safe and effective clinical translation (Table 2).

Clinical Trials of Regulatory T Cell Therapy

Early *in vitro* investigations of Treg therapy in SOT were essential for promoting efficient translation toward clinical implementation. Optimized manufacturing protocols can now generate up to 2×10^9 highly purified Tregs (>90% $CD4^+CD25^{high}CD127^{low}FOXP3^+$) within a 2-week culture period [61]. *In vitro* studies relevant to clinical translation have shown that darTregs exhibit superior suppressive capacity compared with polyclonal Tregs, enabling effective immunoregulation at lower cell doses [62–64]. *In vivo* studies in immunocompetent mice further demonstrated that HLA-A2–targeted CAR-Tregs preferentially accumulate within $HLA\text{-}A2^+$ allografts, highlighting their antigen-specific homing ability [65]. Boardman et al. [66] further suggested that such selective migration significantly enhances the protective efficacy of CAR-Tregs and

Table 2. Clinical trials of Treg therapy in solid organ transplantation on clincialtrials.gov

Organ	Study ID	Acronym	Cell type[a)]	Phase	Cell dose	Location
Kidney	NCT06552169	RETIRE	Polyclonal Tregs	II	-	Taiwan, U.S.
Kidney	NCT02091232	ONE Study	Belatacept-conditioned Tregs	I	1.0×10^6	U.S.
Kidney	NCT03867617	-	Polyclonal with donor bone marrow	I, II	1.0×10^7	Austria
Kidney	NCT02129881	OneTreg1	Polyclonal Tregs	I, II	$1\text{-}10 \times 10^6$/kg	U.K.
Kidney	NCT06777719	Eight Treg	Polyclonal Tregs	I	2.096×10^6 CD8+ Tregs/kg	France
Kidney	NCT02371434	ONEnTreg13	Polyclonal Tregs	I, II	0.5, 1.0, and 2.5-3 × 10^6/kg	Germany
Kidney	NCT01446484	-	Polyclonal Tregs	I, II	2.0×10^8	Russia
Kidney	NCT04817774	STEADFAST	CAR-Tregs	I, II	10^4 to 10^9 cells/kg	Belgium, Netherlands, U.K.
Kidney	NCT02244801	DART	Donor-alloantigen-reactive Tregs	I	3.0 and 9.0×10^8	U.S.
Kidney	NCT02145325	TRACT	Polyclonal Tregs	I	0.5, 1.0, and 5.0×10^9	U.S.
Kidney	NCT02088931	TASKp	Polyclonal Tregs	I	3.2×10^8	U.S.
Kidney	NCT02711826	TASK	Polyclonal Tregs	-	$550\pm450 \times 10^6$	U.S.
Liver	NCT02474199	ARTEMIS	Donor-alloantigen-reactive Tregs	I, II	4×10^8	U.S.
Liver	NCT02188719	deLTa	Donor-alloantigen-reactive Tregs	I	50, 200, 800×10^6	U.S.
Liver	NCT02166177	ThRIL	Polyclonal Tregs	I, II	0.5-1 and 3-4.5 × 10^6/kg	U.K.
Liver	NCT03654040	LITTMUS-UCSF	Polyclonal Tregs	I, II	90 to 500×10^8	U.S.
Heart	NCT04924491	THYTECH	Polyclonal Treg	I, II	1.0×10^7	Spain

Treg, regulatory T cell; CAR, chimeric antigen receptor.
[a)]The source of all cell types was autologous expanded Tregs.

is supported by both CAR- and TCR-mediated reactivity.

After translational studies, initial clinical trials confirmed the safety and feasibility of both expanded polyclonal and darTregs [42,46,56,67]. In 2016, Todo et al. [56] reported the first clinical application of darTregs in living donor liver transplantation, showing that adoptive transfer of *ex vivo*-expanded darTregs was feasible, well tolerated, and permitted complete withdrawal of immunosuppressants in selected recipients. Similarly, the TRACT trial (NCT02145325) in living donor kidney transplant recipients demonstrated that autologous polyclonal Treg infusion was safe, well tolerated, and associated with increased circulating $FOXP3^+$ Tregs, with no rejection episodes or serious infections [68]. These results indicate that Treg therapy has the potential to reduce immunosuppressant requirements, support long-term graft stability, and preserve protective immune responses, thereby laying the foundation for broader clinical use in SOT.

Such encouraging results have been made possible by advances in good manufacturing practice (GMP)-compliant Treg production. For example, at the University of California, San Francisco GMP facility, Tregs were manufactured through a standardized process [45,52]. $CD4^+CD25^+CD127^{low}$ cells were isolated by flow cytometry following sequential magnetic selection and then subjected to a 3-week *ex vivo* expansion with CD3/CD28 antibodies, IL-2, and sirolimus. This approach yielded up to 2×10^9 cells with >90% $FOXP3^+$ purity [52,69,70].

Despite these technical advances, several clinical challenges remain. Key issues include maintaining Treg stability, defining optimal dosing and infusion schedules, and clarifying the influence of immunosuppressive regimens on Treg persistence and function. With respect to stability, the Brunstein trial showed that umbilical cord blood-derived Tregs peaked at day 2 but became undetectable in circulation beyond 14 days. In contrast, the Trzonkowski trial reported a progressive decline in $FOXP3^+$ cells, from 90% to 70% to 40%, at days 75, 82, and 93, respectively, despite three infusions [71,72]. These findings highlight the need to optimize culture duration and stimulation protocols to preserve FOXP3 expression and functional integrity of Treg products. Regarding dosing and infusion schedules, trials have applied a broad range, from 0.43×10^6/kg to 5×10^9 total cells, at different post-transplant time points (e.g., days +10, +13, or +60). These choices are typically influenced by patient immunological risk and individual trial design [45,52,56,68,73]. Furthermore, the choice of immunosuppressive regimen is critical. Rapamycin, for instance, supports Treg survival and expansion more effectively than calcineurin inhibitors,

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while lymphodepletion strategies such as alemtuzumab may enhance Treg engraftment but require careful coordination to avoid unintended depletion [74,75].

For successful clinical translation, harmonized and standardized protocols are essential to optimize outcomes [15]. The ONE Study consortium demonstrated effective multicenter coordination, showing that a single infusion of autologous Tregs in kidney transplant recipients was safe, maintained stable graft function, and reduced the incidence of viral infections [54,76]. In parallel, validated biomarkers are needed for reliable monitoring of therapeutic efficacy. Advances in high-resolution technologies are expected to deepen understanding of Treg heterogeneity and mechanisms of action [77]. Ultimately, the development of robust, scalable, and antigen-specific Treg products will be critical for realizing the full therapeutic potential of Tregs in transplantation.

MONITORING OF REGULATORY T CELLS

The successful application of Treg therapy in SOT requires accurate enumeration and functional assessment of Tregs. Comprehensive monitoring before, during, and after therapeutic infusion depends on highly sensitive and specific qualitative and quantitative assays. Multiple analytical platforms have been developed, including cell-based, protein-based, and gene-based assays. Each provides distinct insights into Treg phenotype and functional activity, including suppression of proliferation, modulation of activation markers, and regulation of cytokine production (Fig. 2).

Cell-Based Phenotypic and Functional Assays

Flow cytometry remains the most widely used cell-based assay for evaluating Treg phenotypes. Phenotypically, Tregs are defined by expression of CD4, CD25, and the transcription factor FOXP3, along with reduced CD127 expression [14]. To address phenotypic heterogeneity, standardized flow cytometry panels incorporating CD3, CD4,

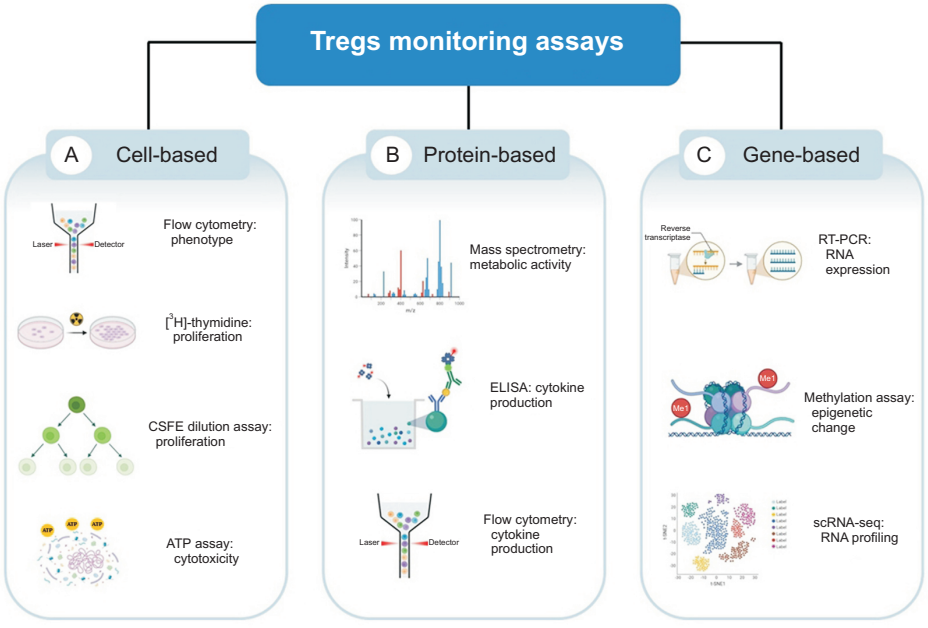


Fig. 2. Analytical approaches used to assess regulatory T cell (Treg) phenotype and function. (A) Cell-based assays include flow cytometry for phenotypic characterization, [^{3}H]-thymidine incorporation, and carboxyfluorescein succinimidyl ester (CFSE) dilution assays for evaluating proliferative suppression, and ATP-based assays for cytotoxic activity. (B) Protein-based assays such as mass spectrometry provide insights into metabolic activity, while enzyme-linked immunosorbent assays (ELISA) and intracellular cytokine staining by flow cytometry are used to quantify cytokine production. (C) Gene-based assays include reverse transcription polymerase chain reaction (RT-PCR) for RNA expression analysis, methylation assays to detect epigenetic modifications (e.g., FOXP3 Treg-specific demethylated region), and single-cell RNA sequencing (scRNA-seq) for high-resolution transcriptional profiling. Together, these complementary methods enable comprehensive monitoring of Treg identity and function in both research and clinical contexts.

CD25, CD127, and FOXP3 have been developed [78]. Additional markers such as Ki67, CD45RA, CTLA-4, and Helios are often included to improve both phenotypic resolution and functional characterization [14,79–82]. The adoption of prealiquoted dried antibody panels and TruCOUNT tubes has enhanced reproducibility and reduced variability across platforms [83,84]. Despite these advances, the absence of fully standardized protocols—particularly regarding gating strategies and interassay consistency—remains a major limitation for broader clinical application.

The suppressive function of Tregs is commonly assessed with *in vitro* proliferation-based assays, such as [^{3}H]-thymidine incorporation and carboxyfluorescein succinimidyl ester (CFSE) dye dilution [85,86]. In the [^{3}H]-thymidine assay, suppression is measured by quantifying radioactive thymidine uptake in $CD4^+CD25^-$ conventional T cells (Tconv) stimulated with anti-CD3 antibodies [87]. This approach is highly sensitive, enabling detection of suppression at low Treg-to-Tconv ratios and providing quantitative proliferation data [85]. However, it underestimates Treg numbers because it cannot distinguish proliferating Tregs from responder T cells within cocultures, and it is further limited by the use of radioactive isotopes and strict timing requirements [88].

The CFSE dye dilution assay allows simultaneous analysis of Tregs and responder T cells, enabling calculation of proliferation indices and suppression rates by multiparameter flow cytometry [89,90]. Nonetheless, this technique is hindered by technical challenges, including substantial cell loss during CFSE staining and reduced signal intensity caused by dye dilution during successive divisions. Importantly, proliferation-based assays such as [^{3}H]-thymidine and CFSE dilution do not assess the ability of Tregs to suppress antigen-specific T cell responses [88].

Protein-Based Phenotypic and Functional Assays

Tregs exert immunosuppressive effects through metabolic disruption and secretion of inhibitory cytokines [29,31,32]. Based on these mechanisms, metabolic profiling and cytokine assays can serve as functional readouts. Metabolic activity is often assessed by measuring CD39/CD73-mediated adenosine production, using techniques such as flow cytometry, immunostaining, mass spectrometry, and western blotting [91]. However, the immunosuppressive effects of Treg-derived adenosine may be offset by effector T cells expressing the CD26-adenosine deaminase complex, which catalyzes adenosine degradation to inosine [92]. Cytokine assays quantify suppressive cytokines such as TGF-β, IL-10, and IL-35 using enzyme-linked immunosorbent assay (ELISA) or flow cytometry [93,94]. ELISA remains the gold standard for cytokine quantification, offering high sensitivity for single or multiplex detection [95]. However, ELISA is limited by difficulties in producing reliable standard curves across wide dynamic ranges and by risks of cross-reactivity, both of which affect accuracy and reproducibility [96]. Its greatest limitation is the inability to identify the specific cell types responsible for cytokine production [97]. In contrast, flow cytometry enables cytokine detection at the single-cell level within heterogeneous Treg populations [98]. Multiparametric approaches, including intracellular staining, permit simultaneous assessment of multiple cytokines, thereby generating a more comprehensive functional profile.

Gene-Based Molecular Assays

The suppressive functions of Tregs are largely determined by the expression of key genes, notably *FOXP3* and *CTLA-4* [99,100]. Transcriptional profiling of these and other Treg-associated genes can be performed using reverse transcription polymerase chain reaction or RNA sequencing [101,102]. Chauhan et al. [103] demonstrated that Tregs with high *FOXP3* expression more effectively suppressed effector T cell proliferation and produced increased IL-10 and TGF-β. In contrast, Zheng et al. [104] reported that while both *FOXP3* and *CTLA-4* are upregulated upon activation, suppressive function correlates more strongly with *CTLA-4* than with *FOXP3*. Importantly, some studies have shown that activated non-Tregs can transiently express *FOXP3* without acquiring suppressive activity [105]. Thus, *FOXP3* alone is insufficient to distinguish bona fide Tregs from activated non-Tregs. Epigenetic modifications, such as DNA methylation patterns in the *FOXP3* Treg-specific demethylated region (TSDR), provide a more reliable means of distinguishing Tregs from other T cell subsets [106–109]. These differences can be quantified by sequencing-based assays, which calculate a demethylation index by comparing the number of demethylated TSDR copies with the total amplified TSDR copies [110,111].

More recently, single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool for tracking immune cell dynamics in tolerance and rejection after Treg therapy in SOT [112,113]. This technology enables high-resolution profiling of infused Tregs and recipient immune cells,

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identifying transcriptional changes, activation states, and lineage heterogeneity. Clinical application of scRNA-seq is expected to provide deeper mechanistic insights into graft-host immune interactions, elucidate therapeutic effects, and facilitate discovery of novel biomarkers for monitoring Treg therapy.

LIMITATIONS OF REGULATORY T CELL THERAPY

The clinical translation of Treg therapy in SOT faces several major challenges. First, the phenotypic and functional heterogeneity of Treg populations complicates standardization of therapeutic products and undermines consistency in suppressive function [114]. Orozco et al. [13] emphasized that variable Treg subsets and unstable *FOXP3* expression impair phenotypic stability and functional capacity. Similarly, Raffin et al. [12] highlighted that Treg instability and plasticity under inflammatory conditions raise concerns regarding both safety and efficacy, as unstable Tregs may convert into pathogenic effector T cells. Another challenge is the potential for resistance to Treg-mediated suppression. In autoimmune diseases, specific effector T cell subsets have been shown to evade Treg regulation, contributing to disease progression [115]. This observation suggests that resistance to Treg-mediated suppression may also occur within the inflammatory environment of SOT and should be carefully considered when applying Treg therapy clinically. A further limitation is the absence of validated biomarkers for *in vivo* persistence, migration, and functional activity. Without reliable biomarkers, monitoring infused Tregs and optimizing therapeutic protocols remain difficult, hindering assessment of efficacy [116]. To address these challenges, harmonized multiomic biomarker approaches are needed. These include *FOXP3* TSDR demethylation to evaluate stability, single-cell transcriptomic and epigenomic profiling for subset characterization, and TCR repertoire analysis for lineage tracking. Such integrated strategies can precisely identify stable and suppressive Treg subsets while enabling real-time monitoring after infusion. Next-generation monitoring will therefore be indispensable for developing robust, safe, and clinically effective Treg therapies in SOT.

CONCLUSION

As Treg therapy progresses toward broader clinical application in SOT, reliable monitoring of Treg phenotype and function has become increasingly crucial. Given the inherent cellular heterogeneity, potential lineage instability, and dynamic functional states of Tregs, conventional monitoring approaches are insufficient for assessing therapeutic efficacy *in vivo*. Accurate assessment tools, including cell-based, protein-based, and gene-based assays, are essential not only to evaluate efficacy and ensure safety but also to guide individualized immunomodulatory strategies. In the absence of standardized monitoring frameworks, interpretation of clinical outcomes and refinement of therapeutic protocols remain limited. Therefore, the development and implementation of comprehensive, sensitive, and clinically validated monitoring strategies must be prioritized. Such efforts will be critical to fully realize the therapeutic potential of Treg therapy in transplantation.

ARTICLE INFORMATION

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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Author Contributions
All the work was done by Minjeong Nam.

REFERENCES

1. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol 1999;163:5211–8.
2. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–61.
3. Grover P, Goel PN, Greene MI. Regulatory T cells: regulation of identity and function. Front Immunol

2021;12:750542.
4. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133:775–87.
5. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008;8:523–32.
6. Ruiz R, Kirk AD. Long-term toxicity of immunosuppressive therapy. In: Busuttil RW, Klintmalm GB, eds. Transplantation of the liver. 3rd ed. Elsevier; 2015. p.1354–63.
7. Lee AY, Jeong J, Heo KN, Park S, Ah YM, Han JM, et al. Complications associated with immunosuppressive agents in solid organ transplant recipients: a nationwide analysis. J Clin Med 2025;14:3602.
8. Khan MA. T regulatory cell mediated immunotherapy for solid organ transplantation: a clinical perspective. Mol Med 2017;22:892–904.
9. Feng G, Wood KJ, Bushell A. Interferon-gamma conditioning ex vivo generates CD25+CD62L+Foxp3+ regulatory T cells that prevent allograft rejection: potential avenues for cellular therapy. Transplantation 2008;86:578–89.
10. Veerapathran A, Pidala J, Beato F, Yu XZ, Anasetti C. Ex vivo expansion of human Tregs specific for alloantigens presented directly or indirectly. Blood 2011;118:5671–80.
11. Yu S, Fu B, He X, Peng X, Hu A, Ma Y, et al. Antigen-specific T-regulatory cells can extend skin graft survival time in mice. Transplant Proc 2011;43:2033–40.
12. Raffin C, Vo LT, Bluestone JA. Treg cell-based therapies: challenges and perspectives. Nat Rev Immunol 2020;20:158–72.
13. Orozco G, Gupta M, Gedaly R, Marti F. Untangling the knots of regulatory T cell therapy in solid organ transplantation. Front Immunol 2022;13:883855.
14. Santegoets SJ, Dijkgraaf EM, Battaglia A, Beckhove P, Britten CM, Gallimore A, et al. Monitoring regulatory T cells in clinical samples: consensus on an essential marker set and gating strategy for regulatory T cell analysis by flow cytometry. Cancer Immunol Immunother 2015;64:1271–86.
15. de Wolf AC, Herberts CA, Hoefnagel MH. Dawn of monitoring regulatory T cells in (pre-)clinical studies: their relevance is slowly recognised. Front Med (Lausanne) 2020;7:91.
16. Huang Y, Shan J, Zhang C, Zhang J, Feng L, Li S, et al. Peripheral blood T regulatory cell counts may not predict transplant rejection. BMC Immunol 2010;11:40.
17. Klein L, Khazaie K, von Boehmer H. In vivo dynamics of antigen-specific regulatory T cells not predicted from behavior in vitro. Proc Natl Acad Sci U S A 2003;100:8886–91.
18. Huang SW, Jiang W, Xu S, Zhang Y, Du J, Wang YQ, et al. Systemic longitudinal immune profiling identifies proliferating Treg cells as predictors of immunotherapy benefit: biomarker analysis from the phase 3 CONTINUUM and DIPPER trials. Signal Transduct Target Ther 2024;9:285.
19. Amini L, Kaeda J, Fritsche E, Roemhild A, Kaiser D, Reinke P, et al. Clinical adoptive regulatory T cell therapy: state of the art, challenges, and prospective. Front Cell Dev Biol 2022;10:1081644.
20. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. Front Immunol 2012;3:51.
21. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 1998;188:287–96.
22. de la Rosa M, Rutz S, Dorninger H, Scheffold A. Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. Eur J Immunol 2004;34:2480–8.
23. Höfer T, Krichevsky O, Altan-Bonnet G. Competition for IL-2 between regulatory and effector t cells to chisel immune responses. Front Immunol 2012;3:268.
24. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in Treg cell function. Nat Immunol 2016;17:1322–33.
25. Puccetti P, Grohmann U. IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. Nat Rev Immunol 2007;7:817–23.
26. Stone TW, Williams RO. Modulation of T cells by tryptophan metabolites in the kynurenine pathway. Trends Pharmacol Sci 2023;44:442–56.
27. Karman J, Jiang JL, Gumlaw N, Zhao H, Campos-Rivera J, Sancho J, et al. Ligation of cytotoxic T lymphocyte antigen-4 to T cell receptor inhibits T cell activation and directs differentiation into Foxp3+ regulatory T cells. J Biol Chem 2012;287:11098–107.
28. Liang B, Workman C, Lee J, Chew C, Dale BM, Colonna L, et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. J Immunol 2008;180:5916–26.

https://doi.org/10.4285/ctr.25.0040

29. Wakamatsu E, Machiyama H, Toyota H, Takeuchi A, Hashimoto R, Kozono H, et al. Indirect suppression of CD4 T cell activation through LAG-3-mediated trans-endocytosis of MHC class II. Cell Rep 2024;43:114655.
30. Sharpe AH. Mechanisms of costimulation. Immunol Rev 2009;229:5–11.
31. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. Immunology 2006;117:433–42.
32. Sullivan JA, Tomita Y, Jankowska-Gan E, Lema DA, Arvedson MP, Nair A, et al. Treg-cell-derived IL-35-coated extracellular vesicles promote infectious tolerance. Cell Rep 2020;30:1039–51.
33. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity 2011;34:566–78.
34. Sojka DK, Fowell DJ. Regulatory T cells inhibit acute IFN-γ synthesis without blocking T-helper cell type 1 (Th1) differentiation via a compartmentalized requirement for IL-10. Proc Natl Acad Sci U S A 2011;108:18336–41.
35. Luo S, Larson JH, Blazar BR, Abdi R, Bromberg JS. Foxp3+CD8+ regulatory T cells: bona fide Tregs with cytotoxic function. Trends Immunol 2025;46:324–37.
36. Cao X, Cai SF, Fehniger TA, Song J, Collins LI, Piwnica-Worms DR, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity 2007;27:635–46.
37. Bolivar-Wagers S, Larson JH, Jin S, Blazar BR. Cytolytic CD4+ and CD8+ regulatory T-cells and implications for developing immunotherapies to combat graft-versus-host disease. Front Immunol 2022;13:864748.
38. Ren X, Ye F, Jiang Z, Chu Y, Xiong S, Wang Y, et al. Involvement of cellular death in TRAIL/DR5-dependent suppression induced by CD4(+)CD25(+) regulatory T cells. Cell Death Differ 2007;14:2076–84.
39. Garín MI, Chu CC, Golshayan D, Cernuda-Morollón E, Wait R, Lechler RI, et al. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. Blood 2007;109:2058–65.
40. Terry LV, Oo YH. The next frontier of regulatory T cells: promising immunotherapy for autoimmune diseases and organ transplantations. Front Immunol 2020;11:565518.
41. Cassano A, Chong AS, Alegre ML. Tregs in transplantation tolerance: role and therapeutic potential. Front Transplant 2023;2:1217065.
42. Chandran S, Tang Q, Sarwal M, Laszik ZG, Putnam AL, Lee K, et al. Polyclonal regulatory T cell therapy for control of inflammation in kidney transplants. Am J Transplant 2017;17:2945–54.
43. MacDonald KN, Piret JM, Levings MK. Methods to manufacture regulatory T cells for cell therapy. Clin Exp Immunol 2019;197:52–63.
44. He X, Smeets RL, van Rijssen E, Boots AM, Joosten I, Koenen HJ, et al. Single CD28 stimulation induces stable and polyclonal expansion of human regulatory T cells. Sci Rep 2017;7:43003.
45. Balcerek J, Shy BR, Putnam AL, Masiello LM, Lares A, Dekovic F, et al. Polyclonal regulatory T cell manufacturing under cGMP: a decade of experience. Front Immunol 2021;12:744763.
46. Tang Q, Lee K. Regulatory T-cell therapy for transplantation: how many cells do we need? Curr Opin Organ Transplant 2012;17:349–54.
47. Tahvildari M, Omoto M, Chen Y, Emami-Naeini P, Inomata T, Dohlman TH, et al. In vivo expansion of regulatory T cells by low-dose interleukin-2 treatment increases allograft survival in corneal transplantation. Transplantation 2016;100:525–32.
48. Govender L, Wyss JC, Kumar R, Pascual M, Golshayan D. IL-2-mediated in vivo expansion of regulatory T cells combined with CD154-CD40 co-stimulation blockade but not CTLA-4 Ig prolongs allograft survival in naive and sensitized mice. Front Immunol 2017;8:421.
49. Aoyama A, Klarin D, Yamada Y, Boskovic S, Nadazdin O, Kawai K, et al. Low-dose IL-2 for in vivo expansion of CD4+ and CD8+ regulatory T cells in nonhuman primates. Am J Transplant 2012;12:2532–7.
50. Brook MO, Hester J, Petchey W, Rombach I, Dutton S, Bottomley MJ, et al. Transplantation Without Over-immunosuppression (TWO) study protocol: a phase 2b randomised controlled single-centre trial of regulatory T cell therapy to facilitate immunosuppression reduction in living donor kidney transplant recipients. BMJ Open 2022;12:e061864.
51. Alzhrani A, Bottomley M, Wood K, Hester J, Issa F. Identification, selection, and expansion of non-gene modified alloantigen-reactive Tregs for clinical ther-

apeutic use. Cell Immunol 2020;357:104214.
52. Putnam AL, Safinia N, Medvec A, Laszkowska M, Wray M, Mintz MA, et al. Clinical grade manufacturing of human alloantigen-reactive regulatory T cells for use in transplantation. Am J Transplant 2013;13:3010–20.
53. Hu M, Hawthorne WJ, Nicholson L, Burns H, Qian YW, Liuwantara D, et al. Low-dose interleukin-2 combined with rapamycin led to an expansion of CD4+CD25+FOXP3+ regulatory T Cells and prolonged human islet allograft survival in humanized mice. Diabetes 2020;69:1735–48.
54. Guinan EC, Contreras-Ruiz L, Crisalli K, Rickert C, Rosales I, Makar R, et al. Donor antigen-specific regulatory T cell administration to recipients of live donor kidneys: a ONE Study consortium pilot trial. Am J Transplant 2023;23:1872–81.
55. Hu M, Rogers NM, Li J, Zhang GY, Wang YM, Shaw K, et al. Antigen specific regulatory T cells in kidney transplantation and other tolerance settings. Front Immunol 2021;12:717594.
56. Todo S, Yamashita K, Goto R, Zaitsu M, Nagatsu A, Oura T, et al. A pilot study of operational tolerance with a regulatory T-cell-based cell therapy in living donor liver transplantation. Hepatology 2016;64:632–43.
57. Muckenhuber M, Wekerle T. T regulatory cell therapy: the price of specificity. Am J Transplant 2023;23:1824–5.
58. Tuomela K, Salim K, Levings MK. Eras of designer Tregs: harnessing synthetic biology for immune suppression. Immunol Rev 2023;320:250–67.
59. Gille I, Claas FH, Haasnoot GW, Heemskerk MH, Heidt S. Chimeric antigen receptor (CAR) regulatory T-cells in solid organ transplantation. Front Immunol 2022;13:874157.
60. Eskandari SK, Daccache A, Azzi JR. Chimeric antigen receptor Treg therapy in transplantation. Trends Immunol 2024;45:48–61.
61. Marín Morales JM, Münch N, Peter K, Freund D, Oelschlägel U, Hölig K, et al. Automated clinical grade expansion of regulatory T cells in a fully closed system. Front Immunol 2019;10:38.
62. Sagoo P, Ali N, Garg G, Nestle FO, Lechler RI, Lombardi G, et al. Human regulatory T cells with alloantigen specificity are more potent inhibitors of alloimmune skin graft damage than polyclonal regulatory T cells. Sci Transl Med 2011;3:83ra42.
63. Suchin EJ, Langmuir PB, Palmer E, Sayegh MH, Wells AD, Turka LA, et al. Quantifying the frequency of alloreactive T cells in vivo: new answers to an old question. J Immunol 2001;166:973–81.
64. Tsang JY, Tanriver Y, Jiang S, Xue SA, Ratnasothy K, Chen D, et al. Conferring indirect allospecificity on CD4+CD25+ Tregs by TCR gene transfer favors transplantation tolerance in mice. J Clin Invest 2008;118:3619–28.
65. Sicard A, Lamarche C, Speck M, Wong M, Rosado-Sánchez I, Blois M, et al. Donor-specific chimeric antigen receptor Tregs limit rejection in naive but not sensitized allograft recipients. Am J Transplant 2020;20:1562–73.
66. Boardman DA, Philippeos C, Fruhwirth GO, Ibrahim MA, Hannen RF, Cooper D, et al. Expression of a chimeric antigen receptor specific for donor HLA class I enhances the potency of human regulatory T cells in preventing human skin transplant rejection. Am J Transplant 2017;17:931–43.
67. Sánchez-Fueyo A, Whitehouse G, Grageda N, Cramp ME, Lim TY, Romano M, et al. Applicability, safety, and biological activity of regulatory T cell therapy in liver transplantation. Am J Transplant 2020;20:1125–36.
68. Mathew JM, H-Voss J, LeFever A, Konieczna I, Stratton C, He J, et al. A phase I clinical trial with ex vivo expanded recipient regulatory T cells in living donor kidney transplants. Sci Rep 2018;8:7428.
69. Hoffmann P, Eder R, Kunz-Schughart LA, Andreesen R, Edinger M. Large-scale in vitro expansion of polyclonal human CD4(+)CD25high regulatory T cells. Blood 2004;104:895–903.
70. Putnam AL, Brusko TM, Lee MR, Liu W, Szot GL, Ghosh T, et al. Expansion of human regulatory T-cells from patients with type 1 diabetes. Diabetes 2009;58:652–62.
71. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood 2011;117:1061–70.
72. Trzonkowski P, Bieniaszewska M, Juścińska J, Dobyszuk A, Krzystyniak A, Marek N, et al. First-in-man clinical results of the treatment of patients with graft versus host disease with human ex vivo expanded CD4+CD25+CD127- T regulatory cells. Clin Immunol 2009;133:22–6.
73. Romano M, Fanelli G, Albany CJ, Giganti G, Lombar-

https://doi.org/10.4285/ctr.25.0040

di G. Past, present, and future of regulatory T cell therapy in transplantation and autoimmunity. Front Immunol 2019;10:43.
74. Akimova T, Kamath BM, Goebel JW, Meyers KE, Rand EB, Hawkins A, et al. Differing effects of rapamycin or calcineurin inhibitor on T-regulatory cells in pediatric liver and kidney transplant recipients. Am J Transplant 2012;12:3449–61.
75. Havari E, Turner MJ, Campos-Rivera J, Shankara S, Nguyen TH, Roberts B, et al. Impact of alemtuzumab treatment on the survival and function of human regulatory T cells in vitro. Immunology 2014;141:123–31.
76. Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, et al. Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. Lancet 2020;395:1627–39.
77. Nam HJ, Gong JR, Kim YH, Nguyen-Phuong T, Byun N, Yoon JH, et al. Simultaneous epigenetic and gene expression profiling at single cell resolution uncovers stem-like Treg subsets induced with oligonucleotide expansion in humans. Immune Netw 2024;24:e39.
78. Blinova VG, Zhdanov DD. Many faces of regulatory T cells: heterogeneity or plasticity? Cells 2024;13:959.
79. Cheung J, Zahorowska B, Suranyi M, Wong JK, Diep J, Spicer ST, et al. CD4+CD25+ T regulatory cells in renal transplantation. Front Immunol 2022;13:1017683.
80. Arroyo Hornero R, Betts GJ, Sawitzki B, Vogt K, Harden PN, Wood KJ, et al. CD45RA distinguishes CD4+CD25+CD127-/low TSDR demethylated regulatory T cell subpopulations with differential stability and susceptibility to tacrolimus-mediated inhibition of suppression. Transplantation 2017;101:302–9.
81. Yu WQ, Ji NF, Gu CJ, Wang YL, Huang M, Zhang MS, et al. Coexpression of Helios in Foxp3+ regulatory T cells and its role in human disease. Dis Markers 2021;2021:5574472.
82. Pereira JA, Lanzar Z, Clark JT, Hart AP, Douglas BB, Shallberg L, et al. PD-1 and CTLA-4 exert additive control of effector regulatory T cells at homeostasis. Front Immunol 2023;14:997376.
83. Cossarizza A, Chang HD, Radbruch A, Abrignani S, Addo R, Akdis M, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition). Eur J Immunol 2021;51:2708–3145.
84. Rajagopal D, Tian L, Xiong S, Wang L, Campbell J, Saraiva L, et al. An accurate and rapid single step protocol for enumeration of cytokine positive T lymphocytes. J Immunol Regen Med 2020;9:10.
85. Collison LW, Vignali DA. In vitro Treg suppression assays. Methods Mol Biol 2011;707:21–37.
86. McMurchy AN, Levings MK. Suppression assays with human T regulatory cells: a technical guide. Eur J Immunol 2012;42:27–34.
87. Zhang L, Manirarora JN, Wei CH. Evaluation of immunosuppressive function of regulatory T cells using a novel in vitro cytotoxicity assay. Cell Biosci 2014;4:51.
88. Ganesan N, Ronsmans S, Hoet P. Methods to assess proliferation of stimulated human lymphocytes in vitro: a narrative review. Cells 2023;12:386.
89. Venken K, Thewissen M, Hellings N, Somers V, Hensen K, Rummens JL, et al. A CFSE based assay for measuring CD4+CD25+ regulatory T cell mediated suppression of auto-antigen specific and polyclonal T cell responses. J Immunol Methods 2007;322:1–11.
90. Schneider A, Buckner JH. Assessment of suppressive capacity by human regulatory T cells using a reproducible, bi-directional CFSE-based in vitro assay. Methods Mol Biol 2011;707:233–41.
91. Mandapathil M, Hilldorfer B, Szczepanski MJ, Czystowska M, Szajnik M, Ren J, et al. Generation and accumulation of immunosuppressive adenosine by human CD4+CD25highFOXP3+ regulatory T cells. J Biol Chem 2010;285:7176–86.
92. Mandapathil M, Szczepanski M, Harasymczuk M, Ren J, Cheng D, Jackson EK, et al. CD26 expression and adenosine deaminase activity in regulatory T cells (Treg) and CD4(+) T effector cells in patients with head and neck squamous cell carcinoma. Oncoimmunology 2012;1:659–69.
93. Wu H, Sun HC, Ouyang GF. Effect of T-regulatory cells and interleukin-35, interleukin-10, and transforming growth factor-beta on diffuse large B-cell lymphoma. World J Clin Cases 2023;11:7075–81.
94. Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. J Immunol 2004;172:5213–21.
95. Chiswick EL, Duffy E, Japp B, Remick D. Detection and quantification of cytokines and other biomarkers. Methods Mol Biol 2012;844:15–30.

96. Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA, et al. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. J Gerontol A Biol Sci Med Sci 2008;63:879–84.
97. Ahirwar R, Bhattacharya A, Kumar S. Unveiling the underpinnings of various non-conventional ELISA variants: a review article. Expert Rev Mol Diagn 2022;22:761–74.
98. Qiu JG, Mei XL, Chen ZS, Shi Z. Cytokine detection by flow cytometry. Methods Mol Biol 2014;1172:235–42.
99. Walker MR, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, et al. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. J Clin Invest 2003;112:1437–43.
100. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000;192:303–10.
101. Muthukumar T, Dadhania D, Ding R, Snopkowski C, Naqvi R, Lee JB, et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med 2005;353:2342–51.
102. Bunnag S, Allanach K, Jhangri GS, Sis B, Einecke G, Mengel M, et al. FOXP3 expression in human kidney transplant biopsies is associated with rejection and time post transplant but not with favorable outcomes. Am J Transplant 2008;8:1423–33.
103. Chauhan SK, Saban DR, Lee HK, Dana R. Levels of Foxp3 in regulatory T cells reflect their functional status in transplantation. J Immunol 2009;182:148–53.
104. Zheng Y, Manzotti CN, Burke F, Dussably L, Qureshi O, Walker LS, et al. Acquisition of suppressive function by activated human CD4+ CD25- T cells is associated with the expression of CTLA-4 not FoxP3. J Immunol 2008;181:1683–91.
105. Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol 2007;19:345–54.
106. Polansky JK, Kretschmer K, Freyer J, Floess S, Garbe A, Baron U, et al. DNA methylation controls Foxp3 gene expression. Eur J Immunol 2008;38:1654–63.
107. Floess S, Freyer J, Siewert C, Baron U, Olek S, Polansky J, et al. Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol 2007;5:e38.
108. Piotrowska M, Gliwiński M, Trzonkowski P, Iwaszkiewicz-Grzes D. Regulatory T cells-related genes are under DNA methylation influence. Int J Mol Sci 2021;22
109. Zhang H, Liu L, Li M. Mini-review of DNA methylation detection techniques and their potential applications in disease diagnosis, prognosis, and treatment. ACS Sens 2024;9:1089–103.
110. Metzker M, Shipkova M, von Ahsen N, Andag R, Abe M, Canzler O, et al. Analytical evaluation of a real-time PCR-based DNA demethylation assay to assess the frequency of naturally occurring regulatory T cells in peripheral blood. Clin Biochem 2016;49:1173–80.
111. Wieczorek G, Asemissen A, Model F, Turbachova I, Floess S, Liebenberg V, et al. Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. Cancer Res 2009;69:599–608.
112. Li X, Li S, Wang Y, Zhou X, Wang F, Muhammad I, et al. Single cell RNA-sequencing delineates CD8+ tissue resident memory T cells maintaining rejection in liver transplantation. Theranostics 2024;14:4844–60.
113. Abedini-Nassab R, Taheri F, Emamgholizadeh A, Naderi-Manesh H. Single-cell RNA sequencing in organ and cell transplantation. Biosensors (Basel) 2024;14:189.
114. Sjaastad LE, Owen DL, Tracy SI, Farrar MA. Phenotypic and functional diversity in regulatory T cells. Front Cell Dev Biol 2021;9:715901.
115. Schlöder J, Shahneh F, Schneider FJ, Wieschendorf B. Boosting regulatory T cell function for the treatment of autoimmune diseases: that's only half the battle! Front Immunol 2022;13:973813.
116. Campbell DJ. Control of regulatory T cell migration, function, and homeostasis. J Immunol 2015;195:2507–13.

https://doi.org/10.4285/ctr.25.0040