Engineered Tolerance: Tailoring Development, Function, and Antigen-Specificity of Regulatory T Cells

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Regulatory T cells (Tregs) are potent suppressors of immune responses and are currently being clinically tested for their potential to stop or control undesired immune responses in autoimmunity, hematopoietic stem cell transplantation, and solid organ transplantation. Current clinical approaches aim to boost Tregs in vivo either by using Treg-promoting small molecules/proteins and/or by adoptive transfer of expanded Tregs. However, the applicability of Treg-based immunotherapies continues to be hindered by technical limitations related to cell isolation and expansion of a pure, well-characterized, and targeted Treg product. Efforts to overcome these limitations and improve Treg-directed therapies are now under intense investigation in animal models and pre-clinical studies. Here, we review cell and protein engineering-based approaches that aim to target different aspects of Treg biology including modulation of IL-2 signaling or FOXP3 expression, and targeted antigen-specificity using transgenic T cell receptors or chimeric antigen receptors. With the world-wide interest in engineered T cell therapy, these exciting new approaches have the potential to be rapidly implemented and developed into therapies that can effectively fine-tune immune tolerance.

Keywords: regulatory T cells, chimeric antigen receptors, T cell receptor, IL-2, autoimmunity, transplantation, inflammatory bowel disease, immunotherapy

INTRODUCTION

Regulatory T cells (Tregs) are essential to maintain self-tolerance and dampen immune responses during infection (1, 2). The best characterized subset of Tregs is defined by high expression of CD25 and FOXP3, the master-regulator of their phenotype and suppressive function (3). The critical role of FOXP3 in controlling Treg development and function is illustrated by the study of Tregs from patients with immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome (4). Depending on the specific mutation, IPEX patients may or may not have circulating FOXP3+ T cells, but even if FOXP3+ T cells are present, they are functionally defective due to inadequate FOXP3 transcriptional function (5–7).

Mechanistically, Tregs suppress the proliferation and function of many immune cells, even at very low Treg:effector cell ratios (2). In terms of suppressive pathways, multiple possibilities have
been described, such as immunosuppressive cytokines, contact-
dependent cytotoxicity, metabolic disruption, and suppression
of antigen presenting cells via co-inhibitory molecule expres-
sion. Focusing on human Tregs, there is a dominant role for
CTLA-4 and TGF-β. Monogenic mutations affecting CTLA-4 or
proteins in its pathway affect Treg function (8, 9) and antibodies
that block activation of TGF-β by human Tregs prevent their
ability to control xenogeneic graft-versus-host disease (GVHD)
(10). An additional aspect of Treg mechanisms is their ability
to take on characteristics of other T helper (Th) cells (11, 12)
resulting in sub-specialization and enhanced suppression of
the Th cell subset they mirror (13). Whether or not these sub-
specialized Tregs have unique suppressive mechanisms or are
simply better able to traffic to the relevant sites of inflammation
remains to be defined.

The immunosuppressive properties of Tregs make them
attractive candidates for cellular therapy, particularly for applica-
tion in conditions such as hematopoietic stem cell transplanta-
tion (HSCT), solid organ transplantation, and autoimmunity.
However, harnessing Tregs for this purpose has not been trivial
due to limitations related to cell isolation and expansion. In this
review, we summarize the current state of Treg therapy in the
clinic and discuss how engineering strategies can be used to
improve upon current approaches.

**CURRENT Treg CLINICAL TRIALS**

There are two main approaches to increase Treg numbers and
function: in vivo “boosting” using small molecules or proteins
and adoptive cellular therapy. To date, the most successful strat-
gy to “boost” Treg in vivo is the use of low-doses of IL-2. When
given in limiting concentrations, IL-2 preferentially expands
CD25+ Tregs without significantly affecting cells expressing
low-levels of CD25, such as resting conventional T (Tconv)
cells or NK cells. This concept was first tested for treatment of
hepatitis-C-virus-induced vasculitis where low doses of IL-2
induced an increase in circulating Tregs and clinical improve-
ments in 8 of 10 patients (14). Subsequently, the beneficial effect
of low-dose IL-2 therapy was also observed in GVHD, alopecia
areae, type I diabetes (T1D), and systemic lupus erythematosus
(15–19). However, a cautionary note is that in one study of T1D
where IL-2 therapy was combined with rapamycin, there was an
unexpected expansion of NK cells and worsening of disease (20).
Thus, this approach may need further refinement to reduce the
risk of expanding non-Tregs. Low-dose IL-2 and other strategies
for in vivo-boosting of Tregs are discussed extensively in Zhang
et al. and Boyman et al. (21, 22).

An alternate to in vivo-boosting is adoptive therapy with
ex vivo-enriched, often expanded, Tregs. This method aims to
overcome defective or low numbers of Tregs by transfer of a large
number of Tregs to re-set the Treg:Tconv cell balance. Adoptive
Treg therapy has been applied in the clinic for many years. The
first successful study reported that chronic GVHD patients treated
with Tregs had a significant reduction in clinical symptoms and
immunosuppression (23). Subsequently, Treg therapy has been
tested in several other GVHD cohorts, overall showing that infu-
sion of autologous or third party (partially HLA-matched) Tregs
is well tolerated, does not inhibit graft-versus-leukemia, and may
be protective from GVHD (24, 25).

Adoptive transfer of Tregs has also been applied successfully
in autoimmunity and organ transplantation. Children with
T1D who received Tregs showed slowed disease progression
and long-term preservation of residual beta-cells (26, 27).
Adoptive transfer of Tregs in adults with T1D is also well toler-
ated, with evidence that the cells persist long term (>1 year)
(28). A clinical trial of in vitro-expanded naïve Tregs is also
underway in Crohn’s Disease, the first application of FOXP3+
Treg immunotherapy for inflammatory bowel disease (IBD)
(ISRCTN97547683) (29). In addition, several clinical trials are
testing autologous polyclonal or antigen-expanded expanded
Tregs in kidney or liver transplantation; these trials are reviewed
extensively in Ref. (30–33). To date, all of these studies have
shown that adoptive Treg therapy in humans is feasible and
safe, and initial data suggest that this approach may also be
effective.

**ENGINEERING IL-2**

With the early success of low-dose IL-2 therapy as an approach
to expand Tregs in vivo, there are now several efforts to improve
upon this approach by modulating the way IL-2 interacts with its
receptors. One strategy to modulate IL-2 is to use IL-2/anti-IL-2
monoclonal antibody (mAb) combination therapy to form “IL-2
complexes” that enhance the half-life of IL-2 after intravenous
injection and provide preferential selection of certain immune
cell subsets. For example, IL-2 in complex with anti-IL-2 mAbs,
JES6-1A12 (mouse), or 5344 (human), preferentially expands
Tregs, but not other IL-2-dependent cells such as CD8+ T and
NK cells (34). This approach enriches Tregs and treats disease in
several different mouse models (22, 34). In 2015, Spangler et al.
solved the crystal structure of IL-2/JES6-1A12, showing that this
IL-2 complex preferentially binds cells with the trimeric IL-2R
(CD25, CD122, and common gamma chain) and not dimeric
complexes (CD122 and common gamma chain), thus selecting
for Tregs because of their constitutive CD25 expression (35).

Another approach to modulate IL-2 is to directly mutate
IL-2 itself to change how it interacts with its receptor complex.
Specifically, IL-2 “muteins” have alterations in the CD25-
binding domain, thus decrease affinity for CD25, and enabling
preferential binding to dimeric IL-2R complexes and activation
of NK and CD8+ T cells (36–38). There is also much commercial
interest in making IL-2 muteins with the opposite effect: IL-2
muteins that preferentially activate Tregs have led to a $400
million investment from Eli Lilly to Nektar Therapeutics and
$300 million from Celgene to Delinia to develop this technol-
ogy (39).

A final approach to modulate IL-2 signaling is to change
IL-2R’s affinity for IL-2. Specifically, it is well established that
single nucleotide polymorphisms in the CD25 locus are associ-
ated with autoimmunity (40–43). Considering the power of
CRISPR/Cas9 technology, in the future it could be possible
to edit risk alleles of CD25 into protective alleles or otherwise
engineer IL-2 signaling pathways to optimize therapeutic Treg
function (44).
ENGINEERING Tregs WITH FOXP3

A hurdle in Treg therapy is generating sufficient numbers for clinical application (33). Since activated Tconv cells also express CD25 and FOXP3, and downregulate CD127, isolating Tregs on the basis of CD25 and CD127 alone introduces the risk of co-purifying and co-expanding non-Tregs. One way to overcome this limitation is to isolate naive CD45RA+CD25- cells from blood to enrich for a more homogeneous population (45, 46). However, this also significantly decreases the number of cells with which a culture can be started. Another potential solution to this problem is to isolate Tregs directly from the thymus for application as a third party cell therapy (47).

An additional approach is to find a way to engineer the desired Treg product. Indeed, the possibility of engineering Tregs via over-expression of FOXP3 has been considered since its discovery, with multiple studies showing that viral-mediated overexpression of FOXP3 in mouse or human T cells can induce suppressive function (48). Notably, in order to re-program human T cells into Tregs, FOXP3 has to be expressed at high and stable levels (49, 50); Treg suppressive capacity can be quickly reversed upon removal of FOXP3 (51).

Although FOXP3 is the master Treg transcription factor, evidence that its over-expression alone does not fully recapitulate the Treg gene signature led to the search for other co-factors and the discovery that co-expression of other transcription factors is important for full lineage specification (52). A consideration is whether studies which found that FOXP3 expression alone is not sufficient to induce a complete Treg gene signature considered the time that may be required for epigenetic re-programing to take place. Epigenetic modification and the consequent change in expression of other transcription factors is necessary to stabilize Treg phenotype and function (3). Since these epigenetic changes may require multiple rounds of cell division, re-programming Tconv cells into Tregs may not take place in short-term culture. The first application of FOXP3-engineered Treg therapy will likely happen as gene therapy for IPEX. CD4+ short-term culture. The first application of FOXP3-engineered re-programing Tconv cells into Tregs may not take place in epigenetic changes may require multiple rounds of cell division, sary to stabilize Treg phenotype and function (3). Since these to take place. Epigenetic modification and the consequent edited the time that may be required for epigenetic re-programing is whether studies which found that FOXP3 expression alone is not sufficient to induce a complete Treg gene signature considered the time that may be required for epigenetic re-programing to take place. Epigenetic modification and the consequent change in expression of other transcription factors is necessary to stabilize Treg phenotype and function (3). Since these epigenetic changes may require multiple rounds of cell division, re-programming Tconv cells into Tregs may not take place in short-term culture. The first application of FOXP3-engineered Treg therapy will likely happen as gene therapy for IPEX. CD4+ T cells from IPEX patients can be efficiently converted into functional and stable Tregs by FOXP3 gene transfer in vitro (53, 54). Testing these cells in vivo will rigorously determine if they have acquired sufficient Treg function to treat the severe autoimmunity in these patients.

CHIMERIC ANTIGEN RECEPTORS

Another approach to engineer antigen-specific Tregs is to use a CAR technology, an idea borrowed from cancer immunotherapy. CARs were first described by Eshhar et al. in 1993 (63) and now being applied in humans for cancer immunotherapy (64–66). CARs give T cells the B-cell-like ability to bind to antigen in an MHC-independent manner. Additionally, the modular design of CARs allows for customization of specific regions, such as the signaling domains, to tailor the desired response from the engineered cell (67).

Over the last decade, a number of publications demonstrated the utility of CARs in Tregs (56). All reports used a standard second-generation design and included the CD28 co-stimulatory domain (Table 1) (68). Beginning with mouse models in 2008, Elinav et al. used Tregs from a mouse expressing a transgene for a hapten 2,4,6-trinitrophenol (TNP)-specific CAR (69). They found that transgenic TNP-specific CAR Tregs mediated antigen-specific suppression of effector T cells in vitro as well as in vivo resistance to colitis. The same group then demonstrated that the TNP-CAR could be introduced into mouse Tregs using retroviral-mediated gene transfer, giving these cells the ability to protect from disease in vivo in a dose-dependent manner (70). In a similar system, mouse CAR Tregs specific for a different model antigen, carcinoembryonic antigen (CEA), prevented disease in a model of colitis better than CAR Tregs specific for an irrelevant antigen. Importantly, these CEA-CAR Tregs homed to the location of the antigen (71).

Apart from these studies in the context of IBD, there is currently only one other report of mouse CAR Tregs. Specifically, in 2012, Fransson et al. developed a CAR specific for myelin...
oligodendrocyte glycoprotein (MOG), the disease-causing agent for experimental autoimmune encephalomyelitis (EAE) (73). In this study, instead of isolating CD25+FOXP3+ Tregs, lentivirus was used to ectopically express FOXP3 and enforce a Treg phenotype. The resultant MOG-specific CAR Tregs suppressed responder T cell expansion in vitro and reversed symptoms of EAE. Overall, these publications provided important proof-of-concept data supporting the development of CAR Tregs for use in human cells.

Several publications have demonstrated the application of CAR technology to human Tregs. Three reports investigated the utility of expressing a CAR specific for HLA-A*02:01 (A2) to test whether CAR Tregs could be a new approach to control alloreactive T cells that cause rejection in HSCT and solid organ transplantation (74–76). The first publication showed that A2-CAR Tregs are activated and proliferate when stimulated through the CAR via coculture with A2-expressing cells (74). Additionally, A2-CAR Tregs prevented engraftment of A2+ PBMCs and development of xenogeneic GVHD in a humanized mouse model. Two other groups confirmed this approach, showing that A2-CAR Tregs suppress alloimmune responses better than polyclonal Tregs in humanized mouse models of A2+ skin xenografts (75, 76). A2 is an ideal antigen to target with CAR Tregs because it is broadly applicable in the transplant setting due to its high allelic frequency, meaning that a significant proportion of organ transplants could potentially benefit from this therapy (74). Moreover, HLAs in general are likely good targets for CAR Tregs since they are a membrane-bound protein specifically expressed on the transplanted tissues.

### TABLE 1 | Summary of salient details from the current chimeric antigen receptor (CAR) regulatory T cells (Treg) publications.

| Antigen and model disease | CAR structure | Species and expression system | Effects of CAR Treg therapy and points of significance | Reference |
|---------------------------|---------------|-------------------------------|-------------------------------------------------------|-----------|
| TNP | Hinge: CD28 | Mouse | Protect from TNBS colitis | (69) | |
| Colitis | TM: CD28 | Transgene | Bystander suppression of oxazolone-induced colitis | | |
| | Co-stim: CD28 | – CD28 signaling required for CAR Treg function | | | |
| | ITAMs: Fcγ | – In vivo imaging of Treg trafficking to site of inflammation | | | |
| TNP | Hinge: CD28 | Mouse | Ex vivo expansion through cognate antigen | (70) | |
| Colitis | TM: CD28 | In vivo | Protect from TNBS colitis | | |
| Carcinoma | Hinge: IgG-Fc | Human | Suppression of CEA-specific antitumor response in humanized mouse model | (72) | |
| | TM: CD28 | Retovirus | | | |
| | Co-stim: CD28 | | | | |
| | ITAMs: CD3ζ | | | | |
| Myeloid endodicytomal glycoprotein | Hinge: IgG-Fc | Mouse | Dual expression system of FOXP3 and CAR | (73) | |
| Experimental autoimmune encephalomyelitis (EAE) | TM: CD28 | Lentivirus | Reversal of EAE clinical symptoms, given at peak of disease | | |
| | Co-stim: CD28 | | | | |
| | ITAMs: CD3ζ | | | | |
| | Hinge: CD28 | Human | Protect from CEA-CAR T effector cell induced colitis | (71) | |
| CEA | TM: unknown | Retovirus | In vivo imaging of Treg trafficking to site of inflammation | | |
| Colitis | Co-stim: CD28 | – Presence of CAR-specific antibodies correlated with disappearance of CAR Tregs | | | |
| | ITAMs: CD3ζ | | | | |
| | Hinge: CD8α | Human | CAR-stimulated Tregs maintain stable phenotype | (74) | |
| Transplant rejection | TM: CD28 | Lentivirus | Suppression of alloantigen-specific T cells in vitro | | |
| | Co-stim: CD28 | – Prevention of xenogeneic GVHD in vivo | | | |
| | ITAMs: CD3ζ | | | | |
| | Hinge: CD8α | Human | Prevention of skin allograft rejection in humanized mouse model | (75) | |
| Transplant rejection | TM: CD28 | Lentivirus | Partial effect of CAR-lacking CD28 and CD3ζ intracellular signaling domains | | |
| | Co-stim: CD28 | – Prevention of skin allograft rejection in humanized mouse models | | | |
| | ITAMs: CD3ζ | – CAR specificity tested against a panel of HLA-typed cells | | | |
| | Hinge: CD8α | Human | | (76) | |
| Transplant rejection | TM: CD8 | Retovirus | | | |
| | Co-stim: CD28 | | | | |
| | ITAMs: CD3ζ | | | | |
| Factor VIII | Hinge: IgG-Fc | Human | CAR directed against clinically-relevant soluble antigen | (77) | |
| Hemophilia A | TM: CD28 | Retovirus | Suppression of recall antibody responses | | |
| | Co-stim: CD28 | – Direct comparison between CAR and T cell receptor engineered Tregs | | | |
| | ITAMs: CD3ζ | | | | |

A summary of the key features of the types of CARs that have been tested in Tregs. To date all CARs have utilized the CD28 co-stimulatory domain, but there are variations in the hinge and transmembrane (TM) regions employed. CARs containing Immune Tyrosine Activation Motifs (ITAMs) either from the FcR or CD3ζ proteins have been tested. All studies report superior effects of antigen-specific CAR Tregs compared to polyclonal or non-specific CAR Tregs.

4Hinge region presumed to be derived from IgG Fc.

This CAR encoded CD3ζ amino-terminal to CD28.
Yoon et al. reported the characterization of human CAR Tregs that target FVIII, the protein lacking in hemophilia which is immunogenic in patients receiving FVIII replacement therapy (77). Of specific interest from this study is the finding that a CAR specific for soluble antigens is suitable for use in Tregs, widening the possible antigen-targets that could be considered. This study also demonstrated that both T cell and antibody responses can be controlled by CAR Tregs. Also of note is that this study directly compared the effects of TCR versus CAR-engineered Tregs, finding that antibody recall responses were more effectively controlled by TCR-transgenic Tregs. More research is required to explore similarities and differences between TCR- and CAR-activated Tregs to better understand the affinity requirements and limitations of each approach (Table 2).

### HOW MIGHT CAR TREG BEHAVE IN HUMANS?

Many of the fundamental properties of Tregs are similar to Tconv cells so it may be possible to predict some aspects of in vivo Treg behavior on the basis of findings from CAR Tconv cells used in the oncology field. However, Tregs also have many unique properties, such as their strict dependence on other cells for IL-2 and constitutive expression of inhibitory proteins such as CTLA-4 and TGF-β. Thus, there is a need for more detailed studies in animal models to fully appreciate the similarities and differences between the two cell types. For example, will CAR Tregs be able to persist long term even if their antigen is not available? Some research has shown that Tregs have different activation requirements than Tconv cells (62, 78), meaning that optimal proliferation and long-term persistence may require Treg-specific CAR design. Will CAR Tregs traffic to the necessary locations and mediate tolerance? CAR Tconv cells have been found to traffic to the lungs before moving to secondary lymphoid organs and disease sites, delaying their tumor-killing effect (79, 80). If there is similar phenomenon with Tregs then regional cell delivery may be preferred (79). Will CAR Tregs induce tolerance, and if yes, what molecular mechanisms will be necessary? CAR-activated Tregs upregulate CTLA-4, LAP, GARP, and CD39 (74), but it is unknown which pathway(s) are necessary for CAR Treg-mediated suppression. Further, what is the primary target of CAR Treg-mediated suppression? It is unknown whether CAR Tregs suppress immune cells at the site of inflammation, in secondary lymphoid organs, or both. Dissecting the mechanisms important to CAR Treg function may also provide clues as to their primary mode and location of immune suppression. Many of these questions are ideally suited for study in models of transplantation where similar questions with polyclonal or transgenic Tregs have been addressed (55).

### NEXT STEPS: WHERE WILL ENGINEERED TREG THERAPIES GO FROM HERE?

Many clinical trials with low-dose IL-2 therapies, expanded polyclonal and antigen-specific Tregs for use in autoimmune diseases, HSCT and solid organ transplantation are underway (18, 31, 33). While initial reports from these trials show that the treatments are well tolerated, the aggregate safety and efficacy data from each approach will greatly inform future studies. Notably, the possible long-term effects, and in particular the potential risk of cancer and infection, of these treatments will not be known for a significant period of time.

We predict that in the next ~5 years there will be a rapid transition from the rather crude current approaches with unmodified IL-2 and/or polyclonal Tregs to engineered approaches that enable precise control over the desired effect (81). It is likely that, as for low-dose IL-2 and polyclonal Treg therapy, transplantation will lead the way in testing these new engineered approaches. HSCT is a setting with a wealth of experience in using engineered T cells for cancer and it would be a natural transition to test engineered Tregs in this context. Moreover, in solid organ transplantation allogeneic HLA antigens represent an ideal target for antigen-specific Tregs because they are only expressed on the transplanted

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**TABLE 2** Comparison of the benefits and limitations of engineering regulatory T cells (Tregs) to express a defined T cell receptor (TCR) versus chimeric antigen receptor (CAR), see also Harris and Krantz (57).

| TCR | CAR |
|-----|-----|
| **Pros:** | **Pros:** |
| ✓ “Natural” protein; engineered cells should not be immunogenic | ✓ MHC-independent antigen detection of soluble or cell-surface antigens |
| ✓ Recall responses of TCR-transgenic Tregs may be more effective than CAR Tregs | ✓ Modular design enables more precise control over the type of antigen-stimulated response |
| ✓ Designed to detect intracellular antigens | ✓ Hinge region provides flexibility, allowing CARs to bind antigen in a variety of orientations |
| ✓ Low affinity but high antigen sensitivity; fewer number of antigens required for TCR activation than CAR activation | ✓ Higher antigen affinity than TCRs |

**Cons:**

- MHC-dependent peptide detection; each TCR complex has limited patient applicability
- May require a large library of several TCR genes to adequately cover MHC/peptide complexes for one disease
- Mispairing with endogenous TCRs could create new specificities and reduce efficacy
- “Unnatural” peptide sequence; construct may be immunogenic and limit ability to administer repeat doses
- Ability to detect cell-surface antigens may be blocked by the presence of competing soluble antigen
issue, minimizing the risk of off-target suppression (56). Additionally, since solid organ transplant donors and recipients are usually not HLA-matched, there is a large pool of patients that could benefit from this treatment. CAR targets for autoimmunity will be more difficult to identify because there are few truly organ and/or cell-specific antigens that would be suitable CAR targets. This challenge is similar to that faced in oncology, where off-target effects of CAR T cells can have devastating consequences (67, 82). The field of engineered Tregs will benefit greatly from the huge resources being invested into solving this problem in oncology (64–66), creating an ideal landscape to support the rapid development of this next generation of Treg therapies.

REFERENCES

1. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. (2012) 30:531–64. doi:10.1146/annurev-immunol-031210-102326
2. McMurchy AN, Gillies J, Allan SE, Passerini L, Gambineri E, Roncarolo MG, et al. Demethylation analysis of the FOXP3 locus shows quantitative defects in regulating immune responses. J Allergy Clin Immunol. (2015) 136(2):610–7. doi:10.1016/j.jaci.2014.12.009
3. Cuende J, Lienart S, Dedobbeleer O, van der Woning B, De Boeck G, Stockis J, et al. Monoclonal antibodies against GARP/TGF-beta1 complexes inhibit the immunosuppressive activity of human regulatory T cells in vivo. J Immunol. (2015) 195(5):2485–94. doi:10.1182/jimmunol.2014.12.001607

AUTHOR CONTRIBUTIONS

ND, JV-S, and ML reviewed the literature and wrote and revised the manuscript.

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29. Canavan JB, Scotta C, Voskenkamper A, Goldberg R, Elder MJ, Shoval I, et al. Developing in vitro expanded CD45RA+ regulatory T cells as an adoptive cell therapy for Crohn's disease. Gut (2016) 65(4):584–94. doi:10.1136/gutjnl-2014-306099

30. Gliwinski M, Iwaszkiewicz-Zgrzes D, Trzonkowski P. Cell-based therapies with T regulatory cells. BioDrugs (2017) 31:335. doi:10.1007/s42259-017-0228-3

31. Tang Q, Vincenti F. Transplant trials with Treg: perils and promises. J Clin Invest (2017) 127(7):2505–12. doi:10.1172/JCI90598

32. Romano M, Tung SL, Smyth LA, Lombardi G. Treg therapy in transplantation: a general overview. Transpl Int (2017) 30(8):745–53. doi:10.1111/tri.12909

33. Trzonkowski P, Bacchetta R, Battaglia M, Berglund D, Bohnenkamp HR, ten Brinke A, et al. Hurdles in therapy with regulatory T cells. Sci Transl Med (2015) 7(304):304a18. doi:10.1126/scitranslmed.aaa7721

34. Rosato V, Woytschak J, Boyman O. Interleukin-2: biology, design and application. Trends Immunol (2015) 36(12):763–77. doi:10.1016/j.ti.2015.10.003

35. Spangler JB, Tomala J, Luca VC, Jude KM, Dong S, Ring AM, et al. Antibodies to interleukin-2 elicit selective T cell subset potentiation through distinct conformational mechanisms. Immunity (2015) 42(5):815–25. doi:10.1016/j.immuni.2015.04.015

36. Carmenate T, Pacios A, Enamorado M, Moreno E, Garcia-Martinez K, Fuente D, et al. Human IL-2 mutein with higher antimurtrur efficacy than wild type IL-2. J Immunol (2013) 190(12):6230–8. doi:10.4049/jimmunol.1201989

37. Rojas G, Carmenate T, Leon K. Molecular dissection of the interactions of an antimurtrur interleukin-2–derived mutein on a phage display-based platform. J Mol Recognit (2015) 28(4):261–8. doi:10.1002/jmr.2440

38. Mitra S, Ring AM, Amarnath S, Spangler JB, Li P, Wu J, et al. Interleukin-2 activity can be fine tuned with engineered receptor signaling clamps. J Immunol (2015) 194(1):113–24. doi:10.4049/jimmunol.1401145

39. Allen SE, Song-Zhao GX, Abraham T, McMurchy AN, Levings MK. In vitro generation of human T regulatory cells: a general overview. Curr Gene Ther (2017) 946:115–32. doi:10.1007/978-1-62703-128-8_8

40. Allan SE, Alstad AN, Merindol N, Crellin NK, Amendola M, Bacchetta R, et al. Generation of potent and stable human CD4+ T regulatory cells by activation-independent expression of FOXP3. Eur J Immunol (2008) 38(12):3382–9. doi:10.1002/eji.200833837

41. Amendola M, Passerini L, Pucci F, Gentner B, Bacchetta R, Naldini L. Regulated and multiple miRNA and siRNA delivery into primary cells by a lentiviral platform. Mol Ther (2009) 17(6):1039–52. doi:10.1038/mi.2009.48

42. Fu W, Ergun A, Lu T, Hill JA, Haxhinasto S, Fassetts MS, et al. A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. Nat Immunol (2012) 13(10):972–80. doi:10.1038/ni.2420

43. Trzonkowski P, Rossi Mei L, Sartriana C, Foustori G, Bondanza A, Naldini L, et al. Cd4+(+) T cells from IPEX patients convert into functional and stable regulatory T cells by FOXP3 gene transfer. Sci Transl Med (2013) 5(215):215ra174. doi:10.1126/scitranslmed.3007320

44. Passerini L, Santoni di Sio FR, Porteous MH, Bacchetta R. Gene/cell therapy approaches for immune dysregulation polyendocrinopathy enteropathy X-linked syndrome. Curr Gene Ther (2014) 14(6):422–8. doi:10.2174/1566611014100112382

45. Hoenpli RE, MacDonald KG, Levings MK, Cook L. How antigen specificity directs regulatory T cell function: self, foreign and engineered specificity. HLA (2016) 88(1–2):3–13. doi:10.1111/tc.12822

46. Dawson NAJ, Levings MK. Antigen-specific regulatory T cells: are police CARs the answer? Transl Res (2017) 187:53–8. doi:10.1016/j.trsl.2016.09.009

47. Harris DT, Kranz DM. Adaptive T cell therapies: a comparison of T cell receptors and chimeric antigen receptors. Trends Pharmacol Sci (2016) 37(3):220–30. doi:10.1016/j.tips.2015.11.004

48. Brusko TM, Koya RC, Zhu S, Lee MR, Putnam AL, McClaymont SA, et al. Human antigen-specific regulatory T cells generated by T cell receptor gene transfer. PLoS One (2010) 5(7):e11726. doi:10.1371/journal.pone.0011726

49. Kim YC, Zhang AH, Su Y, Rieder SA, Rossi RJ, Etttinger RA, et al. Engineered antigen-specific human regulatory T cells: immunosuppression of FVIII-specific T- and B-cell responses. Blood (2015) 125(7):1107–15. doi:10.1182/blood-2014-04-566786

50. Hull CM, Nickolay LE, Estorninho M, Richardson MW, Riley JL, Peafman M, et al. Generation of human islet-specific regulatory T cells by TCR gene transfer. J Autoimmun (2017) 79:63–73. doi:10.1016/j.jaut.2017.01.001

51. Tsang JY, Rataiynoethy L, Li D, Chen Y, Bucy RP, Lau KF, et al. The potency of allospecific Tregs cells appears to correlate with T cell receptor functional avidity. Am J Transplant (2011) 11(8):1610–20. doi:10.1111/j.1600-6143.2011.03650.x

52. Plesa G, Zheng L, Medvec A, Wilson CB, Robles-Oteiza C, Liddy N, et al. TCR affinity and specificity requirements for human regulatory T-cell function. Blood (2012) 119(15):3420–30. doi:10.1182/blood-2011-09-377051

53. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci U S A (1993) 90(2):720–4. doi:10.1073/pnas.90.2.720

54. Maus MV, June CH. Making better chimeric antigen receptors for adoptive T-cell therapy. Clin Cancer Res (2016) 22(8):1875–84. doi:10.1158/1078-0432.CCR-15-1433

55. Esernten JH, Bluestone JA, Lim WA. Engineering therapeutic T cells: from synthetic biology to clinical trials. Annu Rev Pathol (2017) 12:305–30. doi:10.1146/annurev-pathol-052016-100304

56. Sadelain M. Chimeric antigen receptors: driving immunology towards synthetic biology. Curr Opin Immunol (2016) 41:68–76. doi:10.1016/j.coi.2016.06.004

57. Chang ZL, Chen YY. CARs: synthetic immunoreceptors for cancer therapy and beyond. Trends Mol Med (2017) 23(5):430–50. doi:10.1016/j.molmed.2017.03.002

58. Oldham RAA, Medin JA. Practical considerations for chimeric antigen receptor design and delivery. Expert Opin Biol Ther (2017) 17(8):961–78. doi:10.1080/14712598.2017.1339687

59. Elinav E, Adam N, Waks T, Eshhar Z. Amelioration of colitis by genetically engineered murine regulatory T cells redirected by antigen-specific
cMHC-specific chimeric antigen receptor. *Gastroenterology* (2009) 136(5):1721–31. doi:10.1053/j.gastro.2009.01.049
71. Blat D, Zigmond E, Alteber Z, Waks T, Eshhar Z. Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. *Mol Ther* (2014) 22(5):1018–28. doi:10.1038/mt.2014.41
72. Hombach AA, Kofler D, Rappl G, Abken H. Redirecting human CD4+CD25+ regulatory T cells from the peripheral blood with pre-defined target specificity. *Gene Ther* (2009) 16(9):1088–96. doi:10.1038/gt.2009.75
73. Fransson M, Piras E, Burman J, Nilsson B, Essand M, Lu B, et al. CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery. *J Neuroinflammation* (2012) 9:112. doi:10.1186/1742-2094-9-112
74. MacDonald KG, Hoeppli RE, Huang Q, Gallies J, Luciani DS, Orban PC, et al. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. *J Clin Invest* (2016) 126(4):1413–24. doi:10.1172/JCI82771
75. 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(4):931–43. doi:10.1111/ajt.14185
76. Noyan F, Zimmermann K, Hardtke-Wolenski M, Knoefel A, Schulde E, Geffers R, et al. Prevention of allograft rejection by use of regulatory T cells with an MHC-specific chimeric antigen receptor. *Am J Transplant* (2017) 17(4):917–30. doi:10.1111/ajt.14175
77. Yoon J, Schmidt A, Zhang AH, Konigs C, Kim YC, Scott DW. FVIII-specific human chimeric antigen receptor T-regulatory cells suppress T- and B-cell responses to FVIII. *Blood* (2017) 129(2):238–45. doi:10.1182/blood-2016-07-727834

78. Vahl JC, Drees C, Heger K, Heink S, Fischer JC, Nedjic J, et al. Continuous T cell receptor signals maintain a functional regulatory T cell pool. *Immunity* (2014) 41(5):722–36. doi:10.1016/j.immuni.2014.10.012
79. Parente-Pereira AC, Burnet J, Ellison D, Foster J, Davies DM, van der Stegen S, et al. Trafficking of CAR-engineered human T cells following regional or systemic adoptive transfer in SCID beige mice. *J Clin Immunol* (2011) 31(4):710–8. doi:10.1007/s10875-011-9532-8
80. Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanaki J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* (2011) 118(18):4817–28. doi:10.1182/blood-2011-04-348540
81. Lam AJ, Hoeppli RE, Levings MK. Harnessing advances in T regulatory cell biology for cellular therapy in transplantation. *Transplantation* (2017) 101(10):2277–87. doi:10.1097/TP.0000000000001757
82. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics* (2016) 3:16011. doi:10.1038/mto.2016.11

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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