

Review Article



Modulating Autoimmune Diseases with CAR-Tregs: A Cutting-edge Therapeutic Strategy

Abhijit Prasad Mishra¹, Vikram Singh Meena², Pragya Ukey¹, Manish Kumar³ and Sunil Babu Gosipatala^{1*} 

¹Department of Biotechnology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India; ²Department of Zoology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India; ³Centre for Life Sciences, Mahindra University, Hyderabad, Telangana, India

*Correspondence to: Sunil Babu Gosipatala, Department of Biotechnology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India. ORCID: <https://orcid.org/0000-0002-5377-817X>. Tel: +91-9455036926, E-mail: sunil_gos@yahoo.com

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Abstract

Chimeric antigen receptor (CAR)-engineered regulatory T cells (Tregs) have emerged as a groundbreaking advancement in the field of autoimmune disease therapy. By combining the immunosuppressive functions of Tregs with the antigen specificity of CAR technology, CAR-Tregs present a novel strategy to re-establish immune tolerance in an antigen-directed manner. This innovative approach offers a highly targeted and durable alternative to conventional immunosuppressive treatments, which are often limited by systemic toxicity and a lack of long-term efficacy. Preclinical studies in murine models of autoimmune diseases, including multiple sclerosis and type 1 diabetes, have demonstrated the therapeutic potential of CAR-Tregs to achieve localized immune modulation with minimal off-target effects. The suppressive activity of CAR-Tregs is mediated through a multifaceted mechanism involving interleukin-10 and transforming growth factor-beta secretion, cytotoxic-T lymphocyte associated protein 4 engagement, and adenosine production. Despite their remarkable advantages, CAR-Tregs face several challenges, including complexities in manufacturing, difficulty in antigen selection for multifaceted diseases, and potential loss of function over time. This review highlights recent progress in CAR-Treg research, positioning them as a next-generation, precision immunotherapy with the potential to redefine the treatment paradigm for autoimmune disorders. We further summarize the current challenges and advancements in CAR-Treg therapy, followed by a discussion of its future prospects.

Keywords: Adoptive cell therapy; Autoimmune disease; Chimeric antigen receptor-engineered regulatory T cell; CAR-Treg; Chimeric autoantibody receptor T; CAAR-T; Immunotherapy; Antigen specificity; Next-generation therapeutics.

Introduction

Chimeric antigen receptor (CAR)-regulatory T cells (Tregs), which combine the intrinsic stability of Tregs with the engineered antigen specificity of CAR technology, provide a rational and innovative strategy to induce long-lasting immune tolerance. CAR-Tregs represent a promising frontier in cell-based immunotherapy, offering precise and durable immunosuppression for non-malignant immune-mediated conditions such as transplant rejection, autoimmune diseases, and chronic inflammation.¹ Unlike CAR T cells, which are designed to eliminate malignant cells, CAR-Tregs employ targeted immune modulation through antigen-specific suppression, thereby circumventing the systemic immunosuppression and adverse effects commonly associated with conventional therapies.^{2,3} CAR-Tregs are produced by isolating autologous Tregs, genetically engineering them to express CARs, and re-introducing them into the patient. These CARs enable major

histocompatibility complex (MHC)-independent recognition of specific surface antigens, such as proteins, carbohydrates, or glycolipids, facilitating targeted immunosuppression.^{4,5} Upon antigen engagement, CAR-Tregs suppress immune responses through multiple mechanisms, including the secretion of interleukin (IL)-10, transforming growth factor-beta (TGF- β), and IL-35, and through interaction with dendritic cells to inhibit immune activation. Like their effector CAR T cell counterparts, CAR-Tregs demonstrate hallmark features of living therapeutics, including antigen-driven expansion, tissue homing, persistence, and dynamic responsiveness to immune challenges.^{6,7} Preclinical studies have highlighted the therapeutic potential of CAR-Tregs in diverse disease models, including allograft rejection, graft-versus-host disease (GvHD), and autoimmune conditions such as type 1 diabetes (T1D) and multiple sclerosis (MS). In transplantation models, human leukocyte antigen (HLA)-specific CAR-Tregs have extended graft survival by

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promoting antigen-specific tolerance. In T1D models, GAD65-targeted CAR-Tregs effectively trafficked to pancreatic islets and maintained immunoregulatory function for extended periods post-infusion.⁸ Although they failed to prevent disease onset in non-obese diabetic/Ltj mice, their persistence in secondary lymphoid organs reflected robust survival and immunomodulatory capacity. *In vitro*, insulin-specific CAR-Tregs retained a suppressive phenotype and inhibited effector T-cell proliferation, reinforcing their potential for antigen-directed immune regulation.⁹ Further validation comes from inflammatory bowel disease models, where TNP-specific CAR-Tregs conferred antigen-dependent and dose-responsive suppression of colitis, affirming their capacity for *in vivo* expansion and functional adaptability.¹⁰ Similarly, A2-CAR-Tregs ameliorated alloimmune-mediated skin injury, while AAV-CAR-Tregs maintained Treg phenotypes, dampened effector T-cell activity, and attenuated inflammation through sustained cytokine secretion and enhanced transgene expression.¹¹ In addition to direct antigen recognition, CAR-Tregs exhibit bystander suppression, attenuating unrelated immune responses in the surrounding microenvironment. They further promote infectious tolerance by inducing a regulatory phenotype in neighboring immune cells, thereby amplifying and prolonging their immunosuppressive effects.¹² Through these interconnected mechanisms, CAR-Tregs deliver precision immunomodulation. Nevertheless, one of the principal challenges in CAR-Treg therapy is maintaining phenotypic stability. Inflammatory milieus can downregulate forkhead box protein P3 (FOXP3) expression, predisposing Tregs to transdifferentiate into pro-inflammatory effector T cells.¹³ To address this, strategies such as FOXP3 overexpression and careful antigen selection are under investigation. Furthermore, CAR-Treg manufacturing is technically complex, requiring the isolation of highly pure Treg populations and preservation of their functional identity following genetic modification.⁶ Despite these hurdles, CAR-Tregs offer considerable translational promise. Their capacity to induce targeted, long-lasting immune tolerance while preserving systemic immune competence represents a major advancement over existing immunosuppressive therapies. Ongoing innovations in CAR design, gene delivery systems, and safety controls continue to accelerate the clinical translation of CAR-Tregs for autoimmune diseases, transplantation tolerance, and gene therapy applications.¹⁴ Ultimately, CAR-Tregs signify a paradigm shift in the treatment of immune-mediated disorders, moving from generalized immunosuppression to personalized, antigen-specific immune modulation. As living therapeutics, they offer the potential to reshape immune tolerance strategies and revolutionize the management of complex immune-driven diseases.

This review aims to comprehensively examine the evolution, mechanisms, clinical progress, and future prospects of CAR-Treg therapy as a next-generation approach for restoring immune tolerance in autoimmune and inflammatory diseases. Beginning with the conceptual foundation derived from CAR-T cell therapy, we discussed how the success of CAR-T cell technology in oncology has inspired the engineering of Tregs for targeted immune suppression. The review further outlines the molecular design of CAR constructs, preclinical and clinical developments, mechanisms of action, and therapeutic applications across diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), T1D, MS, and Crohn's disease.

Engineering CAR-Tregs: Insights from CAR T cell platforms

The evolution of CAR T cell therapy has laid a critical foundation for the development of CAR-Tregs. While CAR T cells aim to kill cancer cells and CAR-Tregs aim to suppress immune responses, both use similar CAR design, manufacturing methods, and translational approaches.^{4,15}

At the molecular level, both CAR T cells and CAR-Tregs utilize a modular receptor architecture consisting of an extracellular single-chain variable fragment (scFv) for antigen recognition, a hinge and transmembrane domain, and intracellular signaling components. The linker connecting the scFv to the activation domain is typically rich in glycine and serine residues to confer structural flexibility.¹⁶ While CD3 ζ is a common activation motif across both platforms, the selection of co-stimulatory domains is tailored to the therapeutic intent. In CAR T cells, co-stimulatory signals such as CD28 or 4-1BB (CD137) are incorporated to enhance cytotoxicity, persistence, and proliferation. In contrast, CAR-Tregs predominantly favor CD28, as it reinforces FOXP3 expression and supports their regulatory phenotype and suppressive function.⁴

Since their inception, CAR T cell therapies have undergone iterative refinements through successive generations, each improving structural design, signaling fidelity, and therapeutic efficacy.⁴ As living therapeutics, CAR T cells exhibit high antigen specificity, prolonged persistence, and robust clinical responses. In patients with B-cell malignancies—including acute lymphoblastic leukemia, diffuse large B-cell lymphoma, follicular lymphoma, and multiple myeloma—CAR T cells have been detected in peripheral circulation for three to six months post-infusion, demonstrating their capacity for long-term immune surveillance.^{17,18} These clinical successes culminated in the U.S. Food and Drug Administration approval of six CAR T cell therapies beginning in 2017, marking a watershed moment in cancer immunotherapy.⁶ Products approved by the U.S. Food and Drug Administration, such as Kymriah (tisagenlecleucel), Yescarta (axi-cel), Tecartus (brexu-cel), Breyanzi (liso-cel), and Abecma (ide-cel), primarily target CD19 or BCMA antigens and offer transformative, and in some cases curative, treatment options for patients with relapsed or refractory hematologic malignancies.¹⁹ Ongoing clinical trials continue to expand the therapeutic horizon of CAR T cells, while simultaneously informing the design, optimization, and clinical translation of CAR-Tregs in immune-mediated disorders (Table 1).²⁰

Manufacturing CAR T cell therapies presents notable challenges. In lymphogenic and heavily pretreated patients, T cell collection is often difficult, and the prolonged production timeline frequently necessitates bridging chemotherapy.²¹ Furthermore, the autologous nature of these therapies entails substantial costs and intricate logistics, hindering widespread accessibility and clinical implementation.⁴ The integration of chemokine receptors such as C-X-C chemokine receptor type 3 (CXCR3) or C-C chemokine receptor type 4 (CCR4) into CAR-Tregs significantly enhances their trafficking to inflamed tissues, thereby augmenting their immunosuppressive function.^{22,23} In contrast to conventional CAR T cells, CAR-Tregs demonstrate a superior safety profile, with no reported instances of tumor lysis syndrome or cytokine release syndrome. Furthermore, the incorporation

Table 1. FDA-approved CAR T cell-based therapies²⁰

Therapy	Target antigen	Target approved indication	Month and year of approval	Clinical trials	Adverse effects
Kymriah (Tisagenlecleucel)	CD19	Pediatric/ young adult B-ALL; adult DLBCL	August, 2017	NCT02435849 (ELIANA trial); NCT02445248 (JULIET trial)	CRS, Neurotoxicity, B-Cell aplasia
Yescarta (Axicabtagene ciloleucel)	CD19	DLBCL, PMBCL, FL	October, 2017	NCT02348216 (ZUMA-1); NCT03105336 (ZUMA-5)	CRS, Neurotoxicity
Tecartus (Brexucabtagene autoleucel)	CD19	MCL, adult B-ALL	July, 2020	NCT02601313 (ZUMA-2); NCT02614066 (ZUMA-3)	CRS, Neurotoxicity
Breyanzi (Lisocabtagene maraleucel)	CD19	DLBCL, FL, CLL/SLL	February, 2021	NCT02631044 (TRANSCEND-NHL-001); NCT03331198 (TRANSCEND-CLL-004)	Lower rates of CRS and Neurotoxicity
Carvykti (Ciltacabtagene autoleucel)	BCMA	Multiple myeloma	February, 2022	NCT03548207 (CAR-TITUDE-1)	CRS, Neurotoxicity, Delayed Cytopenia
Abecma (Idecabtagene vicleucel)	BCMA	Multiple myeloma (relapsed/refractory after ≥2 prior therapies)	March, 2021	NCT03361748 (KarMMa)	CRS, Neurotoxicity

Clinical trial identifiers refer to registered studies on ClinicalTrials.gov. Common adverse effects of CAR T cell therapies include cytokine release syndrome (CRS) and neurotoxicity, which require specialized management for optimal patient outcomes. B-ALL, B-cell acute lymphoblastic leukemia; BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; FDA, U.S. Food and Drug Administration; FL, follicular lymphoma; MCL, mantle cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; SLL, small lymphocytic lymphoma.

of safety mechanisms, including suicide genes or reversible “OFF” switches, enables precise control over their activity and facilitates their selective elimination when required.²² CAR-Tregs, characterized as CD4⁺ Helios⁺ T cells expressing immunosuppressive markers such as FOXP3 and CD25, play an essential role in modulating immune responses during cancer immunotherapy, particularly in the context of CD19-targeted CAR T cell therapy for large B-cell lymphoma. Detected as early as day 7 post-infusion, these cells are associated with reduced neurotoxicity and delayed disease progression, largely due to their regulatory effect on CAR T cell expansion, especially within the CD8⁺ subset. Notably, integrating CAR-Treg frequency with lactate dehydrogenase levels, a surrogate marker of tumor burden, improves the prediction of clinical outcomes. This combination offers a valuable biomarker for refining therapeutic strategies and managing toxicity in patients with large B-cell lymphoma.²⁴

In contrast to effector CAR T cells that promote immune activation, CAR-Tregs utilize an immunosuppressive mechanism to maintain immune balance and control pathological responses.²⁵ This key distinction underpins their therapeutic promise in settings such as transplantation and autoimmune diseases. However, advancing their clinical application remains challenging, particularly due to the lack of standardized protocols for activation, expansion, and the reliable establishment of a stable regulatory phenotype.²²

Tregs and the emergence of CAR-Treg therapy in autoimmune disease

Tregs are a specialized subset of CD4⁺ T lymphocytes characterized by the expression of the transcription factor FOXP3,

and they play an indispensable role in maintaining immune tolerance and homeostasis.^{26,27} Tregs suppress autoreactive immune cells and prevent aberrant immune activation through multiple mechanisms: secretion of immunoregulatory cytokines such as TGF-β, IL-10, and IL-35; engagement of CTLA-4 to inhibit co-stimulatory signaling; and metabolic disruption via high-affinity CD25-mediated sequestration of IL-2, thereby depriving effector T cells of critical growth signals.²⁸ In autoimmune diseases, diminished Treg frequency or functional impairment permits the unchecked activity of autoreactive lymphocytes, culminating in chronic inflammation and tissue damage.²⁹ Additionally, Tregs can modulate antigen-presenting cells by downregulating co-stimulatory molecules such as CD80 and CD86, limiting their ability to prime autoreactive T cells.³⁰ CAR-Tregs have emerged as a next-generation, precision immunotherapy for autoimmune and inflammatory diseases. By equipping Tregs with synthetic CARs that target disease-relevant autoantigens, these engineered cells can home to sites of inflammation and re-establish immune tolerance in a highly localized and antigen-specific manner.^{31,32} Preclinical studies in models such as experimental autoimmune encephalomyelitis (EAE)—a surrogate for MS—and colitis have demonstrated their capacity to suppress inflammation and reverse disease phenotypes.³³ In contrast to conventional immunosuppressants, which broadly suppress the immune system, CAR-Tregs provide focused modulation of pathogenic immune responses while preserving overall immune competence.³⁴ Despite the promise of CAR-Treg therapy, several challenges persist, including the optimization of CAR design, enhancement of *in vivo* persistence, and mitigation of phenotypic instability. Phenotypic instability in CAR-Treg therapy is a major challenge since Tregs may lose their suppressive function and

convert into effector T cells. The extent of this instability varies with CAR design, as high-affinity CARs and certain signaling domains (e.g., 4-1BB) may exacerbate instability and promote pro-inflammatory cytokine production. Strategies to enhance CAR-Treg stability include careful CAR construct selection, use of endogenous Treg populations, optimization of costimulatory signals, and incorporation of safety mechanisms.³⁵ Nevertheless, ongoing clinical trials in conditions such as pemphigus vulgaris and T1D mark a significant shift from broad immunosuppression toward antigen-specific immune reprogramming.^{36,37} Advances in genomics, synthetic biology, and biomaterial engineering are poised to refine CAR-Tregs technology further, enhancing their safety, stability, and therapeutic precision.³⁸ Collectively, these innovations are paving the way for a new era in the treatment of autoimmune and inflammatory diseases—one defined by durable, localized, and highly specific immune regulation.³⁹

Polyclonal Treg cells as cellular therapeutics

Polyclonal Treg cells, defined by their diverse T-cell receptor (TCR) repertoire, have shown significant therapeutic promise in various preclinical disease models and early-phase clinical studies. They have demonstrated effective immune modulation and the ability to promote immune tolerance in murine models of GvHD,^{40,41} SLE, EAE, colitis, and T1D; adoptive transfer of polyclonal Tregs led to reduced pathology, diminished immune cell infiltration, and overall disease amelioration.⁴² Human polyclonal Tregs have also demonstrated protective effects in xenogeneic GvHD models when transferred into immunodeficient mouse strains. These include Rag2^{-/-} γ c^{-/-} mice deficient in both recombination-activating gene 2 and the common gamma chain (IL2rg) and severe combined immunodeficient mice, underscoring their capacity to modulate pathogenic immune responses across species barriers.⁴³

The phenotypic stability and purity of the cell product are critical factors in the success of polyclonal Treg therapy. Enrichment of naïve Treg subsets, identified by markers such as CD62L in mice and CD45RA in humans, has been associated with sustained FOXP3 expression, driven by epigenetic stability, ultimately enhancing their functional performance *in vivo*.⁴⁴ To generate therapeutically relevant quantities of polyclonal Tregs, standard expansion protocols commonly employ anti-CD3/CD28 stimulation in the presence of high-dose IL-2.⁴⁵ The safety and efficacy of these expanded Tregs have been investigated in multiple Phase 1 and 2 clinical trials targeting a variety of conditions, including T1D (ISRCTN06128462, NCT01210664), GvHD (NCT01660607, NCT00602693, NCT02423915), SLE (NCT02428309), Crohn's disease (NCT03185000), and COVID-19 (NCT04468971). Particularly noteworthy is a Phase 1/2 study of Orca-T, a donor-derived, polyclonal Treg-enriched product administered within 60 h of collection without *ex vivo* expansion, which demonstrated a markedly reduced incidence of both acute and chronic GvHD (4–5%) compared to historical rates of 30%–40%. These encouraging outcomes have prompted the initiation of a Phase 3 randomized clinical trial comparing Orca-T with standard-of-care in patients with acute leukemia undergoing allogeneic hematopoietic stem cell transplantation (NCT05316701).⁴³ Despite significant progress, the therapeutic outcomes of

polyclonal Treg therapy remain variable, primarily due to inconsistencies in isolation and expansion protocols employed across different studies.⁴⁶ In addition, the absence of antigen specificity in polyclonal Tregs can constrain their therapeutic efficacy in specific settings, thereby driving the advancement of engineering approaches aimed at improving their target specificity and functional precision.^{47,48}

Antigen-specific Tregs: Enhancing precision in Treg-based immunotherapy

Although polyclonal Treg cells have shown safety and therapeutic potential across various preclinical and clinical contexts, their lack of antigen specificity can result in systemic immunosuppression, often requiring high cell doses to achieve efficacy. The advent of CAR technology, originally developed to enhance T cell targeting in cancer immunotherapy, has introduced a transformative strategy when applied to Tregs. However, employing generated Tregs for the treatment of autoimmune disorders presents a significant drawback. This occurs because induced Tregs are polyspecific, meaning they might inhibit beneficial immune responses when administered to a patient. The initial phase involved generating induced Tregs that precisely targeted a certain autoantigen. Molecular engineering is essential for the development of CAR-equipped Tregs. CARs represent a viable approach for redirecting the specificity of Treg cells.¹⁶

By equipping Tregs with CARs, their immunosuppressive activity can be precisely directed toward specific antigens or tissues. This approach synergizes the natural regulatory function of Tregs with the targeting accuracy of CARs, enhancing their specificity, potency, and durability. CAR-Tregs can be tailored to recognize disease-relevant antigens in autoimmune disorders, donor-specific antigens in transplantation, or tissue-restricted antigens in inflammatory diseases, enabling localized immune regulation while minimizing the systemic effects commonly seen with conventional immunosuppressive therapies.^{2,3}

Engineering of CAR-Tregs for autoimmune disease therapy

TCR-engineered Tregs face limitations due to their dependence on MHC-restricted antigen recognition, which increases the risk of mispairing between endogenous and introduced TCR chains, thereby complicating their clinical utility. Additionally, their survival and suppressive function remain highly dependent on IL-2.⁴⁹ In contrast, CAR-Tregs offer several compelling advantages. Their MHC-independent antigen recognition broadens their applicability, particularly in the contexts of transplantation and autoimmune disease.² CAR-Tregs also display enhanced homing to antigen-expressing tissues, reduced IL-2 dependency, and the ability to proliferate independently, thereby improving their therapeutic efficacy.³ A foundational preclinical study in 2008 demonstrated that CAR-Tregs could localize to inflamed colonic tissue and suppress effector T cell activity in an antigen-specific, MHC-unrestricted manner, resulting in notable improvement of colitis.⁵⁰ Emerging evidence suggests that CAR-Tregs surpass TCR-engineered Tregs in their ability to prevent GvHD, exhibiting greater suppressive potency and more consist-

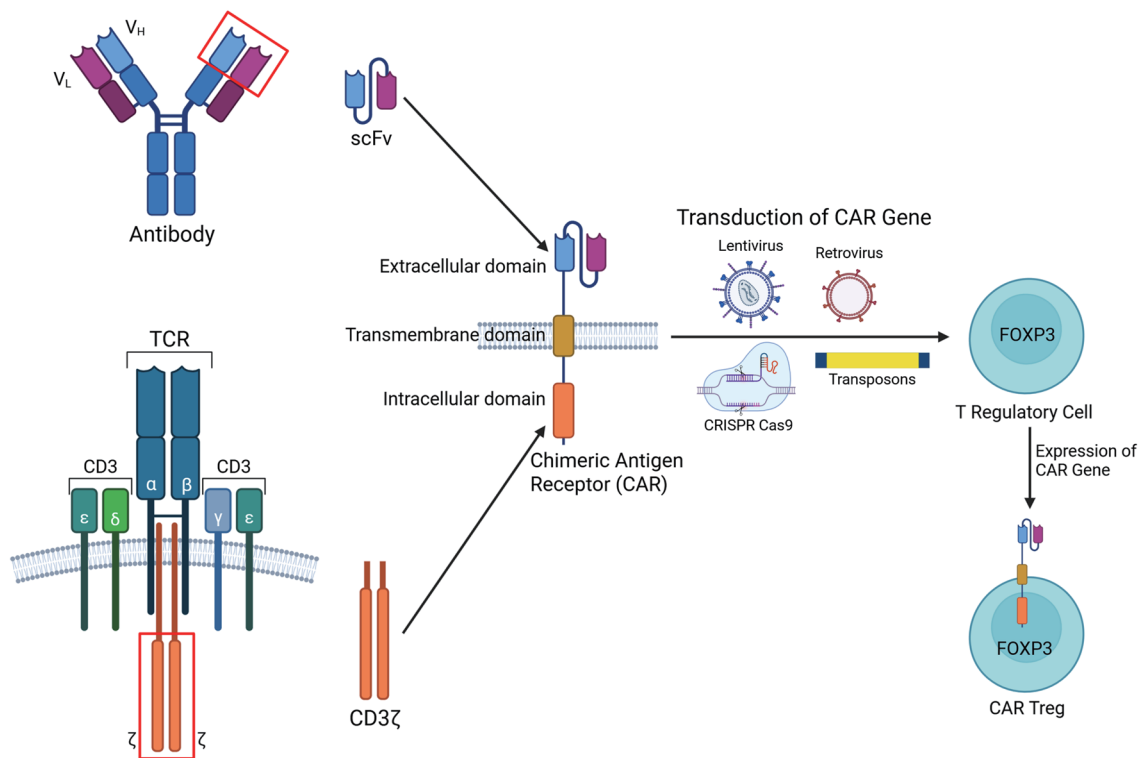


Fig. 1. Construction of chimeric antigen receptor (CAR) regulatory T cells (CAR-Tregs). A chimeric receptor is synthesized by combining the scFv derived from the variable regions of an antibody and the intracellular signaling domain CD3 ζ from the T cell receptor (TCR). The CAR gene is introduced into FOXP3⁺ T regulatory (Treg) cells using viral (lentiviral or retroviral) or non-viral (transposon systems, CRISPR/Cas9) vectors. The result is a CAR-expressing Treg cell with retained immunosuppressive capacity and targeted antigen recognition. The figure was created with BioRender. CRISPR, clustered regularly interspaced short palindromic repeats; FOXP3, forkhead box protein P3; scFv, single-chain variable fragment.

ent therapeutic outcomes.⁵¹ Engineering Tregs with CARs has emerged as a compelling strategy for the treatment of autoimmune diseases. This innovative approach leverages the intrinsic immunosuppressive function of CD4⁺ Tregs while conferring antigen specificity through precise genetic modification, enabling focused immune modulation. Unlike conventional CAR-T cells, which are designed to eliminate malignant targets, CAR-Tregs are programmed to suppress aberrant immune activity at its source. This precision-targeted strategy offers a significant advantage over traditional immunosuppressive therapies by minimizing systemic immune suppression and preserving the body's ability to combat infections.^{52,53} The engineering of CARs for Tregs builds upon foundational CAR T cell technology, with specific modifications tailored to the unique functional and phenotypic characteristics of Tregs. A typical CAR construct consists of three key components: an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain.

In the treatment of autoimmune diseases, CAR-Treg therapy enables precise, antigen-specific immune modulation. The process begins with the isolation of peripheral blood mononuclear cells from sources such as thymus, umbilical cord blood, or peripheral blood.⁵⁴ Tregs are then enriched based on either naïve surface markers (CD4⁺, CD25^{high}, CD127^{low}, and CD45RA⁺), which are associated with superior proliferative potential and phenotypic stability, or conventional markers (CD4⁺, CD25^{high}, CD127^{low}).^{55,56} CAR intro-

duction is typically performed using viral vectors, including lentiviral, gamma-retroviral, or adeno-associated viral systems. However, interest is growing in non-viral gene delivery approaches, such as the Sleeping Beauty transposon system and clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome editing. Notably, CRISPR-engineered CAR-Tregs have shown enhanced durability and functional performance compared to their virally transduced counterparts, highlighting their potential for next-generation cell therapies.³

CAR-Treg construction integrates scFv from monoclonal antibodies to ensure specific antigen recognition. These are fused with co-stimulatory domains, such as CD28 or 4-1BB, which are essential for promoting CAR-Treg persistence, functional stability, and immunosuppressive efficacy.⁵⁷ FOXP3, the master transcription factor critical for sustaining the regulatory identity of conventional T cells, can be upregulated through several approaches (Fig. 1). These include viral transduction, homologous directed recombination, Treg-specific demethylated region demethylation, and inhibition of histone deacetylases.²² Emerging CRISPR/Cas9 gene editing technologies hold significant potential to reinforce Treg stability by upregulating FOXP3 expression. By preserving the immunosuppressive identity of CAR-Tregs, this strategy reduces the likelihood of their transdifferentiation into pro-inflammatory phenotypes, thereby enhancing therapeutic reliability and safety.⁵⁸ CAR constructs can be integrated into

Table 2. CAR-Treg therapies for autoimmune diseases: Preclinical and clinical developments

Disease	Antigen specificity	Results	Clinical trials	References
T1D	Insulin B peptide – MHC class II	Mouse CAR-Tregs prevented adoptive transfer diabetes by BDC2.5 T cells in immunodeficient NOD mice and prevented spontaneous diabetes in wild-type NOD mice	Preclinical	60
T1D	Insulin B peptide – MHC class II	Mouse InsB:R3-CAR-Tregs protected against spontaneous diabetes in NOD.CD28 ^{-/-} mice	Preclinical	61
Multiple sclerosis	Myelin oligodendrocyte glycoprotein (MOG)	MOG-specific CAR-Tregs suppressed CNS inflammation and prevented disease progression in EAE mice modeling multiple sclerosis	Preclinical	32
Rheumatoid Arthritis	Citrullinated proteins	SBT777101, an autologous regulatory CAR-Treg cell therapy targeting citrullinated proteins, was well tolerated with no serious adverse events in patients with moderate-to-severe rheumatoid arthritis	Phase 1 trial (NCT06201416)	62
Crohn's Disease	Interleukin-23 receptor (IL23R)	IL23R-specific CAR-Tregs effectively suppressed intestinal inflammation and restored immune homeostasis in humanized mouse models of Crohn's disease	Preclinical	63
Systemic lupus erythematosus (SLE)	CD19	In a humanized mouse model of SLE, a single infusion of FOXP3-overexpressing, CD19-targeted CAR-Tregs (FOX19 CAR-Tregs) suppressed B cell activity, reduced autoantibody production, and restored immune homeostasis without detectable toxicity	Preclinical	64
IBD	Flagellin	FliC-CAR-Tregs specifically recognize flagellin, migrate to the colon, and enhance immunosuppression and intestinal barrier integrity <i>in vitro</i>	Preclinical	65

"Preclinical" refers to studies conducted in animal models. Results highlight the immunomodulatory effects of CAR-Tregs targeting disease-specific antigens in preclinical trials. CAR, chimeric antigen receptor; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; IBD, inflammatory bowel disease; MHC, major histocompatibility complex; NOD, non-obese diabetic; SLE, systemic lupus erythematosus.

the cells by CRISPR/Cas9, homologous directed recombination, or by using viral vectors.⁵⁹ Table 2 lists the current preclinical and clinical development of CAR-Treg therapy for autoimmune diseases.^{32,60–65} Engineered Tregs can be broadly categorized into existing and emerging CAR-Treg platforms. Current designs include conventional CAR-Tregs, B cell-targeting antibody receptor-Tregs, *FOXP3*-CARs for enhanced phenotypic stability, and universal CAR-Tregs developed for allogeneic applications.^{33,66} Meanwhile, innovative platforms under investigation include Armor-CARs, designed to enhance suppressive function; Tandem CARs, which enable dual-antigen targeting; and SynNotch-CARs (Fig. 2),²² which provide programmable control over CAR expression.^{67,68}

CAR-Tregs: Mechanism of action

CAR-Tregs exert their immunosuppressive effects through a diverse and synergistic array of mechanisms. Upon antigen engagement, they secrete anti-inflammatory cytokines such as IL-10 and TGF- β , fostering a tolerogenic microenvironment that is critical for maintaining immune tolerance.²⁸ Moreover, CAR-Tregs secrete cytotoxic mediators such as granzyme B, enabling the selective elimination of autoreactive effector T cells and thereby reducing inflammation while maintaining protective immunity.⁶⁹ In contrast, their elevated expression of CTLA-4 suppresses co-stimulatory signals on antigen-presenting cells, effectively limiting the activation of

pro-inflammatory T cells.⁶⁹ Furthermore, through the expression of CD25, CAR-Tregs efficiently sequester IL-2, limiting its availability to effector T cells and thus curbing their expansion while sustaining immune tolerance.⁷⁰

One of the key advantages of CAR-Tregs is their ability to target disease-specific antigens, such as myelin basic protein in MS or insulin and GAD65 in T1D, enabling precision targeting without the off-target effects seen in traditional treatments. Preclinical studies have demonstrated that CAR-Tregs engineered to recognize myelin-specific antigens successfully reduce neuroinflammation and demyelination in MS models,⁷¹ while CAR-Tregs targeting pancreatic islet antigens protect β -cells in T1D, thereby preserving insulin production.^{55,72,73} In GvHD, CAR-Tregs targeting alloantigens have been shown to reduce tissue damage while maintaining systemic immunity. This demonstrates CAR-Tregs' capacity to deliver more localized and precise immunosuppression than conventional therapies.⁷⁴

Therapy-induced targets in autoimmune diseases

Type 1 diabetes mellitus (T1DM): Tregs in T1DM cause the immune system to attack and destroy pancreatic β -cells.⁷⁵ To combat autoimmune destruction, CAR-Tregs have been engineered to selectively recognize peptide–MHC class II complexes,⁶⁰ such as Insulin B10–23:I-Ag7 and p63:I-Ag7, which are predominantly expressed in the pancreas

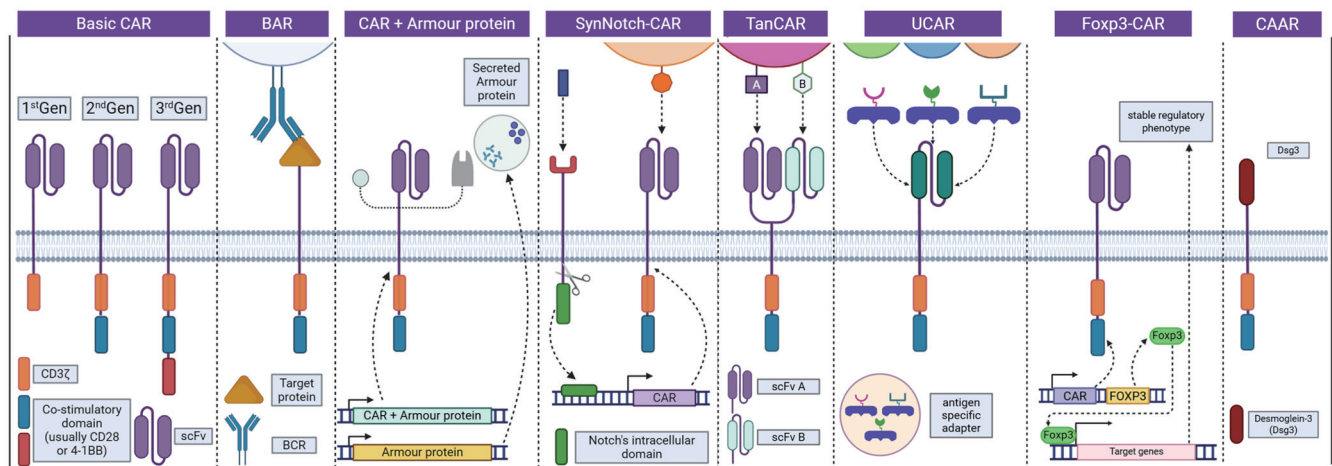


Fig. 2. Structural comparison of chimeric antigen receptor (CAR)-based immunotherapies. Basic CARs (1st–3rd generation) differ by added co-stimulatory domains. Variants enhance function: BAR uses BCR; CAR+Armour secretes modulators; SynNotch-CAR enables conditional CAR expression; TanCAR targets dual antigens; UCAR uses modular adaptors; Foxp3-CAR ensures a stable Treg phenotype. CAAR displays autoantigen (Dsg3) to selectively target autoreactive B cells.²² Figure was created with BioRender. BAR, B-cell receptor antigens for reverse targeting; BCR, B-cell receptor; CAAR, chimeric autoantibody receptor; UCAR, universal chimeric antigen receptor.

and draining lymph nodes.⁷⁶ Whereas autoreactive T cells drive the destruction of β -cells (Fig. 3), CAR-Tregs are programmed to suppress this pathogenic response and attenuate proinflammatory macrophage activity. In comparison with polyclonal Tregs, antigen-specific CAR-Tregs demonstrate superior efficacy in restraining the proliferation of diabetogenic T cells such as BDC2.5 and in reducing inflammatory cytokine production *in vitro*.^{77,78} In animal models, CAR-Tregs targeting InsB10–23:I-Ag7 delayed the onset of diabetes in non-obese diabetic mice and significantly lowered disease incidence relative to controls. The targeted and stable expression of peptide-MHC complexes at sites of autoimmune activity enhances their therapeutic efficacy.^{61,79} These results underscore the potential of CAR-Tregs to precisely regulate immune responses in T1DM through antigen-specific intervention.⁸⁰ **Rheumatoid arthritis:** In Rheumatoid arthritis, compromised Treg function results in elevated levels of proinflammatory cytokines like interferon- γ and tumor necrosis factor- α , intensifying inflammation and promoting joint deterioration.^{81,82} CAR-Tregs are showing great promise as a therapeutic strategy to re-establish immune tolerance in inflamed joints.⁹ Ovalbumin-specific Tregs—whether engineered with TCR alone or with combined TCR and *FOXP3* transduction⁸³—can effectively diminish antigen-driven immune responses in joints by targeting and suppressing inflammatory T helper 17 (Th17) cells, thereby reducing tissue damage. In rheumatoid arthritis, citrullinated vimentin (CV) has emerged as a critical target, leading to the development of CV-specific CAR-Tregs designed to selectively identify and eliminate CV-positive cells in affected joints.^{47,84} Through localized immune suppression, these CV-specific CAR-Tregs offer a potential novel treatment avenue for rheumatoid arthritis.^{82,85,86} To reduce synovial inflammation and protect joint integrity, CAR-Tregs (Fig. 3) act as modulators of aberrant Th17-driven responses,⁸⁷ autoantibody-secreting B cells, and essential inflammatory mediators like IL-17, IL-6, and TNF- α .⁸⁸ Targeting CAR-Treg cells may help regulate the immune system in affected tissues of

rheumatoid arthritis without using broad immunosuppressive therapy.⁸⁹ **SLE:** SLE is a multifaceted autoimmune disorder marked by a loss of immune self-tolerance, primarily driven by overactive B cells,⁹⁰ T cells, and dysregulated innate immune components.⁹¹ This complex immune dysfunction triggers widespread inflammation and the generation of harmful autoantibodies, underscoring the systemic impact of the disease.⁹² Although they require continuous use and pose risks such as infection, modern biologic treatments, including monoclonal antibodies aimed at disease-specific targets, have markedly improved disease control.⁹³ The persistence of autoreactive memory cells, such as plasma cells that continue to generate harmful autoantibodies despite treatment, is a significant problem.⁹⁴ Given the limitations of conventional treatments, CAR-Tregs—engineered Tregs—have emerged as a promising approach to more precisely re-establish immune tolerance in SLE. By specifically targeting HLA-D2/D3 molecules,⁹⁵ CAR-Tregs can inhibit autoreactive B cells and plasma cells responsible for pathogenic autoantibody production, while also suppressing proinflammatory cytokines such as IL-17 (Fig. 3).^{96,97} This targeted strategy avoids the broad immunosuppression associated with traditional therapies, offering the potential for long-term disease control. It reflects a growing shift toward antigen-specific immunomodulation and addresses the limitations of polyclonal Treg therapies, with the goal of achieving sustained remission in SLE patients.^{47,98}

Crohn's disease: Crohn's disease is a chronic autoimmune disorder characterized by transmural inflammation that can involve any segment of the gastrointestinal tract.⁹⁹ The disease arises from a dysregulated immune response, compounded by intricate and imbalanced interactions between the host immune system and the gut microbiota.¹⁰⁰ These maladaptive immune-microbial relationships drive ongoing inflammation and intestinal damage, underpinning the progressive nature of Crohn's disease.^{101,102} Proinflammatory Th17 cells are key contributors to the pathogenesis of Crohn's disease, secreting cytokines such as IL-17, IL-22,

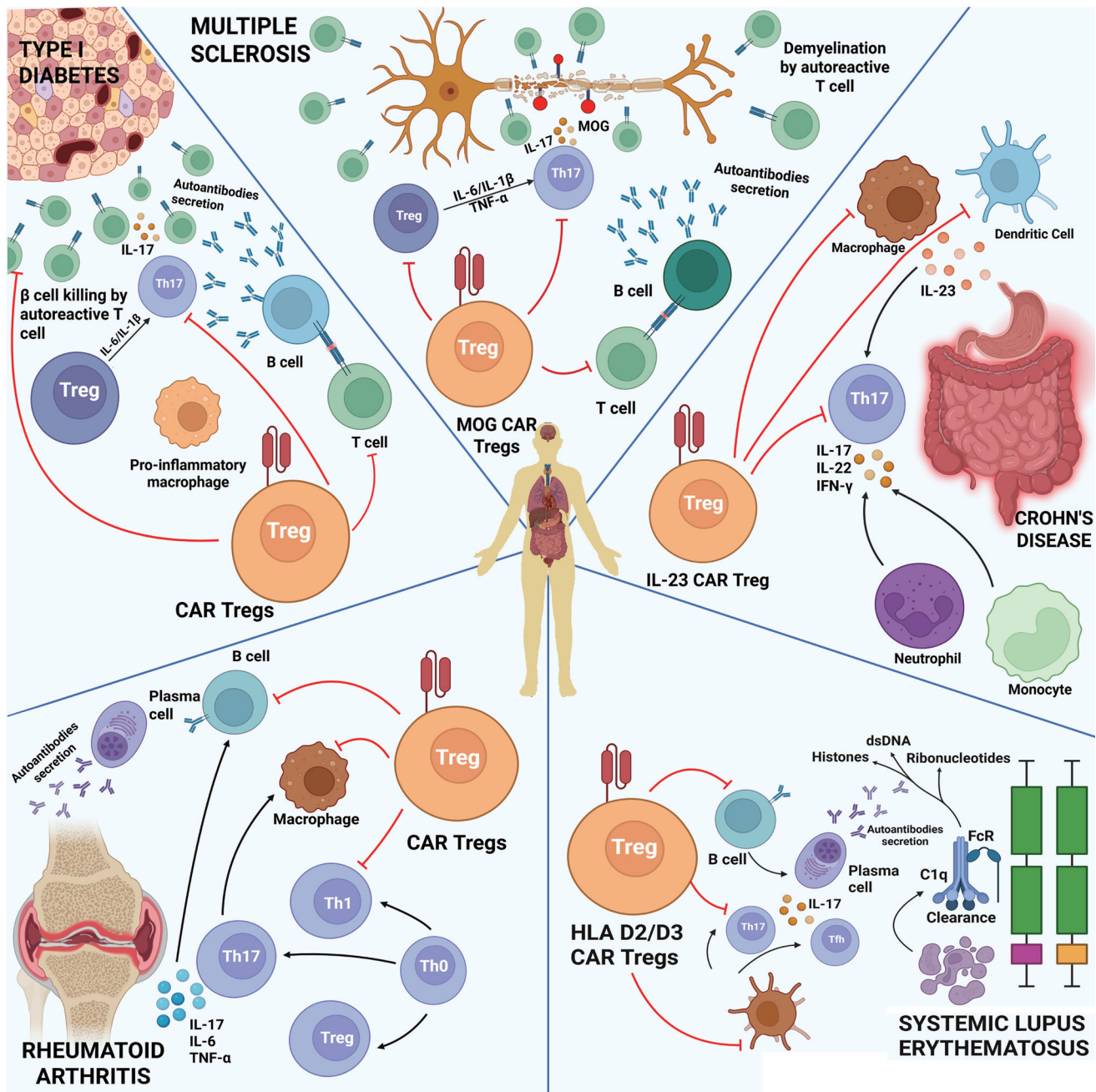


Fig. 3. Engineered chimeric antigen receptor (CAR)-Treg therapy targeting autoimmune disorders. This schematic illustrates how disease-specific CAR-Tregs can modulate immune responses in various autoimmune diseases by suppressing key inflammatory pathways and cell types. In T1D, CAR-Tregs suppress β -cell-targeting T cells and enhance insulin production. In Multiple Sclerosis, MOG-specific CAR-Tregs inhibit demyelinating Th17 responses and B-cell autoantibody production. In Crohn's Disease, IL-23-specific CAR-Tregs dampen Th17-driven gut inflammation and immune cell recruitment. In Rheumatoid Arthritis, CAR-Tregs restore joint homeostasis by suppressing Th17 cells, macrophages, and cytokine release. In Systemic Lupus Erythematosus, HLA-D2/D3-specific CAR-Tregs reduce autoreactive B-cell activity and immune complex formation, limiting systemic inflammation. The figure was created with BioRender. HLA, human leukocyte antigen; MOG, myelin oligodendrocyte glycoprotein; Th17, T helper 17.

and interferon-gamma (IFN- γ) that drive intestinal inflammation and tissue destruction.¹⁰³ This inflammatory cascade is further amplified by dendritic cells and macrophages, which support Th17 cell activation through IL-23 production. CAR-Treg therapies, particularly those targeting the IL-23 recep-

tor (IL-23R), offer a targeted approach to modulating this immune dysregulation.¹⁰⁴ IL-23R CAR-Tregs are designed to home to inflamed intestinal sites, where they suppress Th17-mediated responses, downregulate proinflammatory cytokine release, and inhibit the recruitment of neutrophils and

monocytes. By reestablishing immune tolerance in a highly specific and localized manner, IL-23R CAR-Tregs represent a compelling therapeutic alternative to conventional immunosuppressive therapies (Fig. 3), with the potential to deliver more effective and durable disease control.³ MS: CAR-Treg-cell therapy is gaining recognition as a promising, targeted therapeutic approach for MS—a chronic autoimmune neurodegenerative disease driven by autoreactive T cells and the formation of pathogenic autoantibodies that lead to demyelination and neurological damage.¹⁰⁵ More targeted approaches are required because traditional treatments, such as broad immunosuppressants and B-cell-depleting monoclonal antibodies, can cause systemic immune suppression and adverse effects.¹⁰⁶ CAR-Tregs designed to detect myelin antigens, such as myelin oligodendrocyte glycoprotein,¹⁰⁷ have demonstrated therapeutic potential in light of the functional deficiencies in Tregs in MS patients, including increased IFN- γ and decreased IL-10 production.^{108,109} Antigen-specific CAR-Tregs reduce proinflammatory responses and autoantibody release,¹¹⁰ reducing neuroinflammation through IL-10-dependent mechanisms,¹¹¹ according to preclinical research conducted on EAE models.^{112–114} Notably, myelin basic protein (myelin oligodendrocyte glycoprotein)-targeting altered Tregs effectively relocate to the central nervous system, suppress effector T cells (Fig. 3), and lessen disease severity.^{34,115} Interest in creating CAR-Treg treatments to lower MS-associated morbidity has increased as a result of these developments.

CAR-Tregs clinical trials

Preclinical studies and early-phase clinical trials have demonstrated the safety and promising therapeutic efficacy of CAR-Treg therapy in fostering immune tolerance. In particular, CD45RA⁺ CD19 CAR-Tregs have shown superior functionality, marked by sustained and elevated expression of key regulatory markers such as *FOXP3*, *Helios*, and *CTLA-4*—surpassing the performance of their CD45RO⁺ counterparts.^{22,116} Currently, three clinical trials are actively investigating monospecific CAR-Tregs in human subjects, representing a significant milestone for the field. At present, three clinical trials are actively testing monospecific CAR-Tregs in humans, marking a pivotal milestone in the advancement of the field (Table 3). However, widespread clinical success will depend on continued innovation, including the development of CAR-Tregs capable of targeting multiple antigens to enhance both therapeutic specificity and persistence. Furthermore, the incorporation of ‘armor proteins’ offers a compelling strategy to reinforce functional stability, although comprehensive preclinical evaluation is necessary prior to clinical application.²² Armor proteins include pro-inflammatory cytokines and antibody-like fragments that boost T-cell activity, cancer cell-killing ability, and infiltration.

Advancements in CAR-Treg therapy

Recent advancements in CAR-Treg therapies have significantly expanded their therapeutic potential in the treatment of autoimmune diseases, transplant rejection, and other immune-mediated disorders. A key development is the enhancement of antigen specificity, enabling CAR-Tregs to be

engineered to recognize a broader array of disease-relevant targets, thereby improving their efficacy.¹¹⁷ Additionally, the advent of universal CAR platforms marks a transformative step forward, offering modular systems with interchangeable adapter molecules that allow CAR-Tregs to flexibly and precisely target diverse tissue antigens while preserving their immunoregulatory function and promoting immune tolerance.²² The development of B cell-targeting antibody receptor-Tregs has shown promising results, particularly in controlling severe allergic reactions and modulating excessive antibody responses. Additionally, the CD28 co-stimulatory domain is essential for maintaining CAR-Treg stability and function, helping to prevent premature exhaustion and ensuring long-lasting therapeutic efficacy. The addition of ‘armor’ proteins like IL-2,¹¹⁸ TGF- β , and IL-10,¹¹⁹ has further strengthened the suppressive function, durability, and survival of CAR-Tregs, enhancing their capacity to sustain immune tolerance.

Innovations in orthogonal IL-2 receptors enable the selective expansion of CAR-Tregs, offering precise regulation of cell growth without disrupting the body’s natural immune signaling pathways. Furthermore, CRISPR-Cas9 technology has enabled the precise insertion of CAR constructs into the TRAC locus, ensuring consistent CAR expression while reducing risks such as tonic signaling and Treg exhaustion. These advancements are revolutionizing CAR-Treg therapies, enhancing their stability, efficacy, and adaptability as powerful tools in precision medicine.¹²⁰ CAR-Tregs and chimeric autoantibody receptor (CAAR)-Tregs offer cutting-edge therapeutic approaches for autoimmune diseases by precisely targeting specific antigens to inhibit autoreactive immune responses. In Pemphigus Vulgaris, preclinical research has shown that CAR-Tregs directed against desmoglein 3 (DSG3) can significantly decrease anti-DSG3 antibody production, presenting a non-cytotoxic treatment option. Ongoing clinical trials, such as NCT04422912, are assessing the efficacy of CAAR-Tregs targeting DSG3 in promoting sustained disease remission.²⁰ In Myasthenia Gravis, CAAR-Tregs designed to target muscle-specific tyrosine kinase autoantibodies show promise in selectively suppressing antigen-specific B-cell responses. Ongoing clinical trials, including NCT05451212, are evaluating their effectiveness in improving patient outcomes.²⁰ Similarly, in Neuromyelitis Optica Spectrum Disorders, CAR-Tregs directed against CD19 and CD20 are being evaluated in clinical trials (NCT03605238), aiming to reduce AQP4-IgG levels and alleviate clinical symptoms.²⁰ In idiopathic inflammatory myositis, CAR-Tregs targeting CD19 and CD7 are engineered to regulate B-cell activity and alleviate muscle tissue damage. Likewise, in SLE, CAR-Tregs aimed at CD19 are being developed to deplete autoreactive B cells and diminish autoantibody production.²⁰ Preclinical research on T1D utilizing CAAR-Tregs targeting the I-Ag7-B:9–23 complex has shown promising results in delaying disease onset.²⁰ CAR-Tregs hold significant promise not only for autoimmune diseases but also in transplantation, where they may effectively prevent graft rejection and control GvHD.⁶⁴ The antigen-specific suppressive capabilities of CAR-Tregs significantly enhance immune tolerance toward transplanted tissues, thereby improving both the safety and efficacy of transplantation therapies.¹²¹ Furthermore, their capacity to restore immune homeostasis without inducing broad immu-

Table 3. CAR-Tregs therapies: Registered clinical trials

Clinical trial	Population characteristics	Specifications	Phase of trial	Status	Results	Primary outcomes	Study duration	Clinical trials (NCT ID)
Safety and tolerability of CAR-Treg therapy in living donor kidney transplant patients (STEADFAST)	Living donor renal transplant recipients	HLA-A2 CAR-Tregs, autologous	Phase I/II	Active, not recruiting	Submitted but not available on clinical-trials.gov	This study aims to evaluate the safety and tolerability of TX200-TR101 and its impact on the transplanted kidney in living donor kidney transplant recipients	28 days post infusion	NCT04817774
Safety and clinical activity of QEL-001 in A2-mismatch liver transplant patients (LIBERATE)	A2-mismatch liver transplant patients	HLA-A2 CAR-Tregs, autologous	Phase I/II	Recruiting	No results posted	Long-term safety and Tolerability	28 Days post infusion: from the day of infusion through Week 82 and up to 15 years post-infusion	NCT05234190
Allogeneic CD6-CAR-Tregs for the treatment of patients with chronic graft versus host disease (cGVHD) after allogeneic hematopoietic cell transplantation	Patients with chronic graft versus host disease (cGVHD) after allogeneic hematopoietic cell transplantation	Allogeneic CD6 CAR-Tregs	Phase I	Recruiting	No results posted	Dose-limiting toxicity	From infusion of CD6-CAR- Tregs to day +28	NCT05993611
Long-term follow-up of patients who have received an autologous antigen-specific CAR-Treg therapy (TX200-TR101)	Kidney transplant recipients (18–72 years)	Autologous antigen-specific CAR-Tregs	Phase I/IIa	Enrolling by invitation	No results posted	Safety and tolerability of TX200-TR101 infusion assessed by overall survival and incidence of serious adverse events (SAEs) per CTCAE v5.0	Up to 15 years post-infusion	NCT05987527
First-in-human trial of CAR19 regulatory T cells (CAR19-tTreg) in adults with relapsed/refractory CD19+ B acute lymphoblastic leukemia	Adults with relapsed/refractory CD19+ B-ALL after failure of standard therapies	Allogeneic CAR19 regulatory T cells (CAR19-tTreg)	Phase I/II	Withdrawn (Replaced with a new IND and protocol)	No results posted	Measure CAR19-tTregs efficacy and dose standardization	28 days after CAR19-tTregs administration	NCT05114837
Study of single doses of sbt777101 in subjects with rheumatoid arthritis (Regulate-RA)	Subjects with Rheumatoid Arthritis	SBT777101	Phase I	Recruiting	No results posted	Incidence and nature of dose-limiting toxicities (DLTs)	Day of treatment to end of follow-up (48 weeks). Day of treatment to end of DLT evaluation period (28 days)	NCT06201416

Phase I/II/IIa denotes early human trials evaluating safety. "Phase I/II" indicates trials assessing both safety and preliminary efficacy. Outcome measures, such as dose-limiting toxicity, safety, tolerability, and long-term follow-up, are specific to each study protocol, as noted. Status terms ("Recruiting", "Withdrawn", etc.) reflect current clinical trial enrollment or activity as registered on ClinicalTrials.gov. B-ALL, B-cell acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CTCAE, common terminology criteria for adverse events; HLA, human leukocyte antigen; IND, investigational new drug; SAE, serious adverse event.

nosuppression positions CAR-Tregs as a valuable adjunct in the field of transplantation medicine.⁵⁰

The development of CAR-Tregs and CAAR-Tregs faces several critical challenges. Immunogenicity remains a major concern, as immune responses against CAR components could potentially aggravate autoimmune conditions.^{5,122} Furthermore, manufacturing complexities, including the reliable incorporation of autoantigens and maintenance of Treg regulatory function, continue to hinder clinical translation and large-scale application.^{20,123} In conclusion, CAR-Tregs and CAAR-Tregs hold significant promise as next-generation therapies for autoimmune diseases, offering the potential for targeted and durable immune regulation. Nonetheless, their clinical success hinges on addressing critical challenges such as immunogenicity, efficient and scalable manufacturing, and refined antigen specificity. Resolving these issues will be essential to ensure their safety, efficacy, and broad accessibility in real-world therapeutic settings.¹²⁴

Challenges in CAR-Treg therapy

Although CAR-Treg therapy holds considerable promise, its scalability remains limited by the labor-intensive and costly processes required for Treg isolation and expansion, thereby constraining widespread clinical application. Innovations in bioreactor technologies and the development of off-the-shelf allogeneic CAR-Treg products offer potential solutions to these challenges, paving the way for broader accessibility.⁷¹ Compared to conventional flask-based culture, bioreactors provide an automated system that maintains optimal nutrient supply, oxygenation, and waste removal, in addition to addressing quality control issues. Ongoing clinical trials are currently assessing the safety and efficacy of CAR-Tregs in autoimmune conditions such as T1D and pemphigus vulgaris, providing critical insights into their therapeutic potential in human patients.⁵²

The therapeutic efficacy of Tregs depends on their stability and plasticity. Non-engineered Tregs may transform into proinflammatory Th17 cells under specific conditions, which can compromise their immunosuppressive capabilities.³ Engineered Tregs, such as CAR-Tregs, can achieve enhanced stability through co-transduction with *FOXP3* cDNA, which helps maintain their suppressive phenotype and prevents unwanted lineage diversion.³ Certain CAR-Tregs, particularly those incorporating 4-1BB costimulatory domains, may exhibit reduced stability; however, this limitation can be mitigated through adjunctive therapies such as mTOR inhibitors, which help reinforce their regulatory function.³ In contrast, A2-CAR-Tregs maintain high expression of Treg markers and stable epigenetic features, ensuring robust suppressive function and enhanced targeting capabilities.² Overall, CAR-Tregs demonstrate enhanced stability, antigen specificity, and immunosuppressive capacity compared to polyclonal Tregs, positioning them as a highly promising approach for inducing targeted immune tolerance.

Research underscores the critical role of the CD28 costimulatory domain in boosting CAR-Treg functionality for effective immune suppression. However, challenges such as ligand-independent CAR tonic signaling, which may impair Treg performance, must be resolved to preserve their suppressive ability.⁵¹ Despite these hurdles, both preclinical and clinical data highlight the safety and

effectiveness of CAR-Tregs compared to traditional CAR-T cells (CAR-Tconvs). By selectively suppressing immune responses in lymphoid organs—where antigen presentation and antibody production take place—CAR-Tregs can interrupt the pathological immune activation that drives autoimmune diseases, all while minimizing the risk of systemic immunosuppression.⁶⁴

Transfecting CARs into Tregs is commonly achieved using viral vectors, such as lentiviruses or retroviruses. However, these approaches risk insertional mutagenesis from DNA integration, which may lead to neoplasia.¹²⁵ To improve the safety of CAR-Treg therapies, guided transgene delivery systems offer a promising alternative, enabling safer and more natural CAR expression, for example, by linking the transgene to the TCR promoter while knocking out the TCR.^{126,127} Non-viral methods, such as liposomes and electroporation, are being explored, but their efficiency remains limited.¹²⁸ Currently, CAR-Treg therapies rely on autologous CD4⁺ Tregs, which may be less effective in patients with autoimmune diseases due to Treg dysfunction.^{129,130} Gene editing to remove MHC-related genes could allow the use of allogeneic Tregs, addressing the challenges of defective autologous Tregs in autoimmune conditions.¹³¹

Targeting cells to the specific locations of autoimmune inflammation, such as the synovial joints in rheumatoid arthritis or the pancreatic islets in T1D, is a crucial improvement to CAR-Treg treatment. Following chemokine gradients like C-C motif chemokine ligand 19 (CCL19) / C-C motif chemokine ligand 21 (CCL21), C-C motif chemokine ligand 17 (CCL17) / C-C motif chemokine ligand 22 (CCL22), C-X-C motif chemokine ligand 9/10/11 (CXCL9/10/11), and native T cells use chemokine receptors like CCR7 to move into lymphoid tissues and CXCR3, CCR4, and CCR8 to migrate into peripheral inflammatory regions.¹³² The improved localization, retention, and immunosuppressive action at target tissues are demonstrated by preclinical models of CAR T cells designed to co-express receptors such as CCR7 for lymph node homing and CXCR3 or CCR4 for peripheral movement. CAR-Tregs can accomplish targeted, site-specific immune regulation by utilizing such receptor-directed homing techniques, which increases therapeutic potency while lowering systemic effects.¹³³

Limitations

Despite the growing evidence and encouraging recent progress for CAR-Treg therapy in autoimmune diseases and transplantation, this review has certain limitations. First, most of the available evidence is derived from preclinical murine studies, which may not fully capture the complexity of human immune responses, leaving translational efficacy and safety of CAR-Tregs uncertain. Second, clinical trial data on CAR-Tregs are still sparse, and most ongoing studies are in early-phase evaluation, limiting the ability to draw definitive conclusions on long-term outcomes. Third, the heterogeneity of autoimmune diseases complicates the generalization of CAR-Tregs' therapeutic potential across diverse pathogenic contexts. Finally, this review has primarily focused on scientific and translational perspectives, with less emphasis on economic, ethical, and regulatory considerations, all of which will be crucial for the real-world adoption of CAR-Tregs therapies.

Future implications

CAR-Treg therapy is rapidly emerging as a groundbreaking strategy for the treatment of autoimmune and inflammatory diseases. Originally pioneered for controlling transplant rejection and GvHD, its therapeutic horizon has expanded to encompass complex disorders such as rheumatoid arthritis, SLE, T1D, MS, and ocular conditions like uveitis.⁴¹ By enabling precise, antigen-specific immune suppression, CAR-Tregs offer the unique advantage of long-lasting immune modulation without the collateral damage often associated with broad immunosuppressive therapies. This precision holds the potential to significantly reduce—or even eliminate—the need for lifelong immunosuppressive drugs, thereby lowering the risk of opportunistic infections, malignancies, and other systemic complications.

Recent advancements in CAR design have propelled the field forward. The integration of bispecific CARs, universal adapter-based systems, and sophisticated costimulatory domains such as CD28 has markedly improved the specificity, stability, and functional potency of CAR-Tregs.²² Moreover, cutting-edge genetic engineering, such as the introduction of IL-10-secreting CAR-Tregs or the disruption of exhaustion markers like programmed cell death protein 1, is enhancing both phenotypic stability and suppressive durability.¹³⁴ Innovations in delivery strategies, such as intravitreal administration for localized autoimmune conditions, are opening new therapeutic avenues. Concurrently, the rise of gene-editing technologies like CRISPR and non-viral delivery platforms is facilitating the development of both personalized and allogeneic “off-the-shelf” CAR-Treg products. These off-the-shelf platforms not only offer cost-effective scalability but also promise greater global accessibility—key to making this therapy equitable across diverse healthcare systems.

Despite these strides, important challenges remain. Achieving precise antigen targeting, maintaining long-term Treg stability in inflammatory environments, and scaling manufacturing processes for clinical-grade CAR-Tregs are critical for broader clinical translation. Overcoming these hurdles will be essential to fully unlock the therapeutic promise of this cell-based platform. Looking ahead, CAR-Tregs are poised to redefine the landscape of immunotherapy—not just for autoimmune disease, but also in transplantation medicine and chronic inflammatory conditions. Their ability to restore immune tolerance in a tissue-specific, durable, and non-toxic manner represents a paradigm shift in how we approach immune dysregulation. In summary, CAR-Treg therapy is not just a therapeutic innovation, but a foundational shift toward precision immune modulation. As advancements in CAR engineering, gene editing, and manufacturing continue to evolve, CAR-Tregs are increasingly positioned as the next frontier in immunotherapy, offering tailored, safe, and effective solutions for some of the most challenging immune-mediated diseases.

Conclusions

Building on the clinical success of CAR-T cell therapy, CAR-Treg therapy has emerged as a powerful adaptation of this platform for immune modulation. Early clinical trials such as STEADFAST (NCT04817774) and LIBERATE (NCT05234190) have demonstrated the safety and feasibility

of HLA-A2–specific CAR-Tregs in transplantation, while studies like SBT777101 for rheumatoid arthritis highlight its broader therapeutic promise. These pioneering efforts confirm that CAR-Tregs can induce targeted and durable immune tolerance with minimal systemic toxicity. The integration of gene-editing tools, universal CAR platforms, and bioreactor-based expansion systems will be pivotal for CAR-Tregs’ transition from experimental therapy to a clinically transformative tool—redefining precision immunotherapy for autoimmune diseases and transplant medicine.

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Conflict of interest

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Author contributions

Conception and design, supervision, visualization, manuscript review and editing (SBG), data collection, analysis, drafting of the original manuscript (APM), drafting and critical revision of the manuscript (VSM), drafting of the original manuscript and figure preparation (PU), supervision, visualization, manuscript writing, review, and editing (MK). All authors have read and approved the final manuscript.

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