JOURNAL OF IMMUNOLOGY RESEARCH AND INNOVATION
Jan-Mar 2025, VOL. 2, ISSUE 1, pp. 1-6
https://doi.org/10.61577/jiri.2025.100001

RESEAPRO JOURNALS

REVIEW

OPEN ACCESS

Recalibrating immune balance: CRISPR based reengineering of CTLA-4 and PD-1 pathways

Pratyush Malik

Department of Biotechnology, KIIT University, Bhubaneswar, Odisha

ABSTRACT

CRISPR-based gene editing holds transformative potential for autoimmune disease therapy by precisely modulating immune checkpoints like CTLA-4 and PD-1. These checkpoints are essential for immune regulation, maintaining self-tolerance and preventing excessive immune responses. Dysregulation of CTLA-4 and PD-1 contributes to autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and type 1 diabetes. Current therapies targeting these checkpoints, while effective, often come with limitations, such as off-target effects and systemic immunosuppression. CRISPR-Cas9, along with related technologies like Cas12a and base editing, offers a precise approach to modulate checkpoint expression and restore immune balance. Advances in T cell editing, delivery strategies (viral vectors, electroporation, lipid nanoparticles), and precision techniques are paving the way for safer, more targeted therapies. Preclinical and clinical studies have shown promising results in restoring immune tolerance and enhancing T cell function. However, challenges such as off-target effects, ethical concerns, and regulatory hurdles remain. Future prospects include combining CRISPR with nanotechnology, artificial intelligence, and novel genome-editing tools, offering new avenues for personalized treatments in autoimmune diseases.

KEYWORDS

CRISPR-Cas9; Immune checkpoints; Autoimmune diseases; CTLA-4; PD-1

ARTICLE HISTORY

Received 15 January 2025;
Revised 27 February 2025;
Accepted 21 March 2025

Introduction

Autoimmune disorders, defined by the immune system's aberrant attack on self-tissues, affect millions globally, leading to chronic inflammation and organ damage. Circumstances such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and type 1 diabetes (T1D) exemplify the complexity and heterogeneity of these disorders. Despite advances in immunosuppressive therapies, many patients experience suboptimal responses and adverse effects, underscoring the need for more targeted treatment strategies [1].

Central to immune homeostasis are checkpoint molecules like cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). CTLA-4, stated on regulatory T cells (Tregs) and activated conventional T cells, participates with the co-stimulatory receptor CD28 for binding to CD80/CD86 on antigen-presenting cells, thereby attenuating T cell activation. PD-1, found on T cells, B cells, and myeloid cells, binds to its ligands PD-L1 and PD-L2, carrying inhibitory signals that check immune responses and encourage self-tolerance. Dysregulation of these checkpoints has been concerned in the pathogenesis of various autoimmune diseases, making them attractive therapeutic targets [2]. Monoclonal antibodies targeting CTLA-4 and PD-1 have revolutionized cancer immunotherapy by enhancing antitumor immunity. However, their application in autoimmune diseases is limited due to the risk of exacerbating immune responses and inducing immune-related adverse events. Furthermore, systemic blockade of these checkpoints lacks specificity, potentially disrupting immune tolerance and leading to unintended consequences [3].

The advent of CRISPR-Cas9 genome editing technology offers a promising avenue used for precise modulation of immune checkpoints. By enabling targeted editing of CTLA-4 and PD-1 genes in specific immune cell subsets, CRISPR-Cas9 holds the potential to restore immune balance without the broad immunosuppression associated with current therapies. This approach could lead to personalized treatments that correct underlying immunological defects, offering hope for durable remission in autoimmune diseases [4].

Immunological role of CTLA-4 and PD-1 in Autoimmunity

Mechanisms of immune tolerance mediated by CTLA-4 and PD-1

The immune system employs inhibitory receptors to preserve self-tolerance and prevent autoimmunity. Two pivotal immune checkpoints, Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) and Programmed Death-1 (PD-1), play crucial roles in this regulatory process:

CTLA-4

Expressed on activated T cells and constitutively on regulatory T cells (Tregs), CTLA-4 competes with the co-stimulatory receptor CD28 for binding to B7 molecules (CD80/CD86) on antigen-presenting cells (APCs). Due to its higher affinity, CTLA-4 effectively outcompetes CD28, carrying inhibitory signals that attenuate T cell activation and proliferation, thereby maintaining peripheral tolerance [5].

PD-1

Induced upon T cell activation, PD-1 interacts with its ligands PD-L1 and PD-L2, leading to the recruitment of phosphatases

***Correspondence:** Pratyush Malik, Department of Biotechnology, KIIT University, Bhubaneswar, Odisha, email: 2261091@biotech.kiit.ac.in

that dephosphorylate key signaling molecules downstream of the T Cell Receptor (TCR). This interface results in reduced T cell proliferation, cytokine production, and survival, contributing to the maintenance of immune homeostasis [6].
CTLA-4 and PD-1 regulate T cell activity at different stages and locations in the immune response. These checkpoints are also differentially implicated across autoimmune diseases (Table 1).

Table 1. Key differences between CTLA-4 and PD-1 immune checkpoints.

Feature	CTLA-4	PD-1
Expression Timing	Early activation of T cells	Later stages of T cell activation
Ligands	CD80, CD86	PD-L1, PD-L2
Site of Action	Lymphoid organs	Peripheral tissues
Function	Competes with CD28, inhibits co-stimulation	Suppresses TCR signaling, cytokine release
Autoimmune Relevance	RA, T1D, MS, SLE	SLE, MS, T1D

Dysregulation of CTLA-4 and PD-1 in autoimmune conditions

Aberrations in the expression or function of CTLA-4 and PD-1 have been implicated in the pathogenesis of various autoimmune diseases.

CTLA-4

Genetic polymorphisms in the CTLA-4 gene have been associated with increased susceptibility to autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Type 1 Diabetes (T1D), and Multiple Sclerosis (MS). For instance, the +49 A/G polymorphism has been linked to T1D in certain populations. Moreover, CTLA-4 deficiency or reduced expression can lead to uncontrolled T cell activation and proliferation, contributing to autoimmune pathology [7].

PD-1

Similarly, polymorphisms in the PD-1 gene have been associated with autoimmune conditions. PD-1 deficiency in animal models results in lupus-like symptoms, and reduced PD-1 expression has been observed in patients with SLE and MS. Such dysregulation compromises the inhibitory signaling necessary for maintaining self-tolerance [8].
The loss of functional CTLA-4 and PD-1 pathways disrupts the delicate balance between immune activation and inhibition, leading to the development and progression of autoimmune diseases.

Disease-specific examples

Systemic lupus erythematosus (SLE)

SLE is characterized by the creation of autoantibodies against nuclear mechanisms. Studies have shown that patients with SLE exhibit reduced expression of CTLA-4 and PD-1, correlating with disease activity [7].

Rheumatoid arthritis (RA)

In RA, impaired CTLA-4 function leads to enhanced T cell activation and pro-inflammatory cytokine production, contributing to joint inflammation and destruction. Therapeutic agents like abatacept, a CTLA-4-Ig fusion protein, have been employed to modulate this pathway [8].

Type 1 diabetes (T1D)

T1D involves the autoimmune destruction of pancreatic β-cells. CTLA-4 and PD-1 pathways are crucial in regulating T cell responses against β-cell antigens. Animal studies have demonstrated that blockade of these checkpoints accelerates diabetes onset, highlighting their protective roles [9].

Multiple sclerosis (MS)

MS is an autoimmune disorder targeting the central nervous system. Reduced expression of CTLA-4 and PD-1 has been observed in MS patients, suggesting a breakdown in inhibitory signaling that permits autoreactive T cells to attack myelin sheaths [9].

These examples underscore the significance of CTLA-4 and PD-1 in maintaining immune tolerance and the consequences of their dysregulation in autoimmune pathologies.

CRISPR-Cas Systems: Overview and Advances in Immune Cell Editing

Basic principles of CRISPR-Cas9 and related technologies

The CRISPR-Cas system, derivative from a bacterial adaptive immune mechanism, has revolutionized genome editing. Central to this system is the Cas9 endonuclease, guided by a single-guide RNA (sgRNA) to introduce double-stranded breaks at specific genomic loci. The Streptococcus pyogenes Cas9 (SpCas9) recognizes a 5'-NGG-3' protospacer adjacent motif (PAM) sequence adjacent to the target site. Upon binding, Cas9 induces a double-strand break, which the cell repairs via non-homologous end joining or homology-directed repair, enabling targeted gene modifications [10].

Beyond Cas9, other nucleases like Cas12a (formerly Cpf1) have been identified. Cas12a recognizes a 5'-TTTV-3' PAM sequence and introduces staggered cuts, producing sticky ends, which can be advantageous for certain applications. Notably, Cas12a processes its own CRISPR RNA (crRNA) without the need for a trans-activating crRNA (tracrRNA), simplifying the system. Additionally, base editors, which couple catalytically impaired Cas proteins with deaminases, allow for precise nucleotide conversions without inducing double-stranded breaks, reducing potential off-target effects [11].

A variety of CRISPR systems are being optimized for immune cell editing. Table 2 summarizes key features of Cas9, Cas12a, and base editors in the context of autoimmune gene therapy.

Table 2. Comparison of major CRISPR-based genome editing systems.

Editing Tool	Mechanism	Targeting Features	Use in Immune Cells	Limitations
CRISPR-Cas9	Double-stranded breaks	PAM: NGG	T cells, macrophages	Off-target effects
Base Editors	Single-stranded overhangs	PAM: TTTV	NK cells, T cells	Fewer tools than Cas9
Base Editors	Converts C→T or A→G	No DSB required	Potential for precise edits	Off-target deamination

https://doi.org/10.61577/jiri.2025.100001

Delivery strategies: viral vectors, electroporation, lipid nanoparticles

Efficient delivery of CRISPR components into immune cells is crucial for successful genome editing. Several delivery methods have been developed viral vectors like Adeno-associated viruses (AAVs) and lentiviruses are commonly used for delivering CRISPR components due to their high transduction efficiency. However, concerns regarding immunogenicity, insertional mutagenesis, and limited cargo capacity necessitate alternative approaches [9,12].

Electroporation like physical method uses electrical pulses to transiently permeabilize cell membranes, facilitating the entry of CRISPR ribonucleoprotein (RNP) complexes. Electroporation has been shown to achieve high editing efficiencies in various immune cells, including T cells and hematopoietic stem cells. The technique's parameters, such as voltage and pulse duration, can be optimized to balance efficiency and cell viability. Lipid Nanoparticles LNPs encapsulate CRISPR components, protecting them from degradation and facilitating cellular uptake. This non-viral method offers advantages like reduced immunogenicity and the ability to deliver large payloads. Recent studies have demonstrated the potential of LNPs in delivering CRISPR components to various cell types, including immune cells [13].

Precision and safety: off target effects, pam restrictions, recent improvements

While CRISPR technology offers unparalleled precision, off-target effects remain a concern. Strategies to enhance specificity and safety include, optimizing sgRNA and modifying the length and sequence of sgRNAs can reduce off-target activity. For instance, truncated sgRNAs (17-18 nucleotides) have been shown to decrease unintended edits without compromising on-target efficiency. Engineering High-Fidelity Cas Variants like SpCas9-HF1 and eSpCas9 have been developed to minimize non-specific interactions, thereby reducing off-target cleavage. These engineered nucleases maintain robust on-target activity while enhancing specificity. Expanding PAM Compatibility: Traditional Cas9 recognizes a limited set of PAM sequences, restricting targetable genomic regions. Engineered variants, such as xCas9 and Cas12a variants with altered PAM specificities, have broadened the range of editable sites, enhancing the versatility of CRISPR applications [14].

Chemical Modifications of sgRNAs Incorporating chemical modifications into sgRNAs can improve their stability and reduce off-target effects. For example, 2'-O-methyl and phosphorothioate modifications at specific positions have been shown to enhance specificity and nuclease resistance. Collectively, these advancements in CRISPR technology and delivery methods have significantly improved the precision and safety of genome editing in immune cells, paving the way for therapeutic applications in autoimmune diseases and beyond [15].

CRISPR Editing of CTLA-4 and PD-1: Preclinical and Clinical Insights

The advent of CRISPR-Cas9 technology has revolutionized the field of immunotherapy, offering precise genome editing capabilities that can modulate immune checkpoints such as CTLA-4 and PD-1. These checkpoints play pivotal roles in maintaining immune homeostasis, and their dysregulation is implicated in various autoimmune diseases and cancers. This section delves into the current status of gene editing of CTLA-4 and PD-1 in T cells, explores experimental models and case studies, discusses synergistic approaches combining CRISPR with adoptive T cell therapy, and examines the clinical trials landscape along with translational hurdles [16].

Gene editing of CTLA-4 and PD-1 in T cells

CRISPR-Cas9-mediated gene editing has been employed to disrupt CTLA-4 and PD-1 genes in T cells to enhance their effector functions. For instance, knocking out CTLA-4 in cytotoxic T lymphocytes (CTLs) has been shown to augment their anti-tumor activity. In a study, CTLA-4 knockout CTLs exhibited increased tumor cell killing and elevated secretion of pro-inflammatory cytokines such as TNF-α and IFN-γ. These modified CTLs also demonstrated enhanced tumor control in vivo, indicating the potential of CTLA-4 editing in boosting T cell responses [17].

Similarly, PD-1 disruption in T cells has been explored to counteract T cell exhaustion, a common hurdle in chronic infections and cancer. CRISPR-Cas9-mediated PD-1 knockout in primary human T cells resulted in reduced PD-1 expression and enhanced effector functions, including increased cytokine production and cytotoxicity. These findings underscore the therapeutic promise of targeting PD-1 to rejuvenate T cell responses [18].

Experimental models and case studies

Preclinical studies have provided valuable insights into the effects of CTLA-4 and PD-1 editing. In mouse models, conditional deletion of CTLA-4 in adult mice led to spontaneous lymphoproliferation and organ-specific autoimmunity, highlighting the critical role of CTLA-4 in maintaining immune tolerance. These models serve as essential platforms to study the consequences of checkpoint modulation and to evaluate potential therapeutic interventions [19].

Ex vivo studies using human cells have also been instrumental. For example, gene editing of T cells from patients with CTLA-4 insufficiency restored CTLA-4 expression and function, demonstrating the feasibility of correcting genetic defects through targeted editing. Such approaches pave the way for personalized therapies addressing specific immune dysregulations [18,19].

Synergistic approaches: CRISPR and adoptive T cell therapy

Combining CRISPR-mediated gene editing with adoptive T cell therapy (ACT) has emerged as a promising strategy to enhance therapeutic efficacy. In the context of cancer immunotherapy, CRISPR-Cas9 has been utilized to disrupt PD-1 in tumor-infiltrating lymphocytes (TILs), resulting in improved anti-tumor responses. A study demonstrated that PD-1-deficient TILs exhibited increased cytokine production and cytotoxicity, leading to better tumor control in preclinical models [20].

Moreover, integrating CRISPR editing with chimeric antigen receptor (CAR) T cell therapy has shown potential. By

https://doi.org/10.61577/jiri.2025.100001

knocking out PD-1 in CAR T cells, researchers have enhanced their persistence and anti-tumor activity. Such modifications aim to overcome the immunosuppressive tumor microenvironment and improve the durability of CAR T cell therapies [21].

Clinical trials landscape and translational hurdles

The translation of CRISPR-edited T cells into clinical applications is underway, with several trials assessing their safety and efficacy. In a pioneering study, CRISPR-edited T cells targeting PD-1 were infused into patients with refractory non-small-cell lung cancer. The treatment was generally well-tolerated, with manageable adverse events, and the edited T cells persisted in patients, indicating the feasibility of this approach [21,22].

Despite these advancements, several challenges remain. Ensuring the precision and safety of gene editing is paramount, as off-target effects could lead to unintended consequences. Moreover, the scalability of manufacturing gene-edited T cells and navigating regulatory pathways are significant considerations for broader clinical adoption. Ethical concerns regarding genome editing also necessitate careful deliberation and oversight [23]. CRISPR-Cas9-mediated editing of CTLA-4 and PD-1 in T cells holds substantial promise for enhancing immunotherapeutic strategies. Ongoing research and clinical trials continue to refine these approaches, aiming to translate them into effective treatments for autoimmune diseases and cancers.

Therapeutic Potential and Implications in Autoimmune Diseases

The integration of CRISPR-Cas9 technology into immunotherapy has opened new avenues for treating autoimmune diseases by precisely modulating immune checkpoints such as CTLA-4 and PD-These strategies aim to restore immune tolerance and suppress aberrant immune responses characteristic of autoimmune conditions [24].

One approach involves using CRISPR-Cas9 to knock out inhibitory checkpoints like PD-1 and CTLA-4 in T cells, thereby enhancing their effector functions. This strategy has shown promise in cancer immunotherapy, where disrupting PD-1 expression in CAR T cells leads to improved anti-tumor activity. Conversely, in the context of autoimmunity, the goal is often to enhance the function of regulatory T cells (Tregs) to suppress overactive immune responses. For instance, CRISPR-mediated editing of Tregs to stabilize FOXP3 expression and enhance suppressive capabilities has been explored as a therapeutic avenue. Modulating Treg function through CRISPR-edited CTLA-4 presents another promising strategy. CTLA-4 is constitutively expressed on Tregs and is crucial for their suppressive function. Deficiencies or dysfunctions in CTLA-4 expression can lead to impaired Treg activity and subsequent autoimmunity. CRISPR-Cas9 can be employed to enhance CTLA-4 expression or function in Tregs, thereby restoring their ability to maintain immune homeostasis. For example, editing Tregs to express higher levels of CTLA-4 has been shown to improve their suppressive function in models of autoimmune diseases [25,26].

autoreactive T cells is another area of interest. PD-1 plays a vital role in maintaining peripheral tolerance by inhibiting T cell activation. Targeted modulation of PD-1 expression in autoreactive T cells using CRISPR-Cas9 can potentially reinstate tolerance and prevent autoimmune responses. For instance, studies have demonstrated that depletion of PD-1-expressing cells can induce immune tolerance through peripheral clonal deletion, highlighting the therapeutic potential of PD-1 modulation. The potential to personalize therapies based on disease and checkpoint profiles is a significant advantage of CRISPR-based interventions. Autoimmune diseases are heterogeneous, with variations in immune checkpoint expression and function among individuals. By profiling patients' immune landscapes, therapies can be tailored to target specific checkpoints or pathways involved in their disease. For example, in diseases where Treg dysfunction is prominent, enhancing CTLA-4 expression in Tregs may be beneficial. In contrast, in conditions characterized by hyperactive effector T cells, modulating PD-1 expression could be more effective. This personalized approach ensures that therapies are more effective and have fewer off-target effects [22,25].

CRISPR-Cas9 technology offers a versatile platform for modulating immune checkpoints to treat autoimmune diseases. By precisely editing genes involved in immune regulation, it is possible to restore tolerance and suppress pathological immune responses. Ongoing research and clinical trials will further elucidate the efficacy and safety of these approaches, paving the way for personalized and effective treatments for autoimmune conditions [26].

Ethical, Regulatory, and Safety Considerations

Germline vs. somatic editing in immune modulation

The application of gene editing technologies like CRISPR/Cas9 raises distinct ethical concerns depending on whether germline or somatic cells are targeted. Germline editing involves modifications to reproductive cells (sperm, eggs, or embryos), making changes that are inheritable by future generations. This type of editing is highly controversial due to potential unforeseen genetic consequences, ethical questions about human enhancement, and the risk of misuse. As a result, many countries have implemented strict bans or moratoriums on germline editing in clinical settings. In contrast, somatic editing—where genetic changes are confined to non-reproductive cells—affects only the treated individual and is widely viewed as more ethically permissible. Somatic interventions avoid heritable risks and are generally acceptable under robust ethical oversight, especially when aimed at treating severe or life-threatening conditions [27].

Ethical issues in gene editing for autoimmune diseases

Using CRISPR/Cas9 to treat chronic autoimmune diseases presents several ethical challenges. Informed consent is paramount, as patients must understand both the benefits and the potential risks, including unknown long-term outcomes and the possibility of off-target genetic changes. Another critical concern is equitable access to these advanced therapies. Given their complexity and cost, CRISPR-based treatments may only be available to a limited segment of the population, exacerbating health disparities. Additionally, while early clinical trials have shown promise, the long-term safety of edited immune cells remains uncertain. Potential issues include

https://doi.org/10.61577/jiri.2025.100001

unintended immune responses or the development of secondary diseases, such as cancer, which necessitate prolonged follow-up and monitoring [28].

Regulatory framework and global perspectives

Globally, regulatory agencies are actively working to develop comprehensive frameworks for the clinical use of CRISPR-edited therapies. In the United States, the FDA evaluates these treatments under rigorous guidelines, focusing on safety, efficacy, and ethical integrity. The European Medicines Agency (EMA) follows a similarly cautious approach. Importantly, CRISPR-based therapies have started to reach real-world application; for example, the FDA recently approved the first CRISPR-derived treatment for sickle cell disease, signifying a pivotal step in therapeutic genome editing. Despite this progress, international consensus remains fragmented. While some countries embrace these technologies under controlled conditions, others impose strict limitations or outright bans, reflecting diverse ethical standards and societal views regarding human genetic modification [28,29].

Future Perspectives and Challenges

Emerging tools: CRISPRa/i, prime editing, and epigenome editing

Advancements in CRISPR technology have led to the development of tools like CRISPR activation (CRISPRa) and interference (CRISPRi), which modulate gene expression without altering the DNA sequence. These tools can upregulate or downregulate immune checkpoint genes, offering nuanced control over immune responses in autoimmune diseases.

Prime editing, a newer CRISPR-based technique, enables precise DNA modifications without inducing double-strand breaks. This method holds promise for correcting point mutations associated with autoimmune conditions, potentially restoring normal immune function [17,22]. Epigenome editing, utilizing CRISPR fused with epigenetic modifiers, allows for the reversible regulation of gene expression. This approach can modulate immune-related genes, offering therapeutic avenues for autoimmune disorders without permanent genetic alterations.

Integrating CRISPR with immuno-nanotechnology and AI-guided design

Combining CRISPR with immuno-nanotechnology enhances the delivery and specificity of gene-editing therapies. Nanoparticles can be engineered to target specific immune cells, improving the efficiency and safety of CRISPR-based treatments [29].

Artificial intelligence (AI) is increasingly being employed to design and optimize CRISPR components. AI algorithms can predict off-target effects and guide the development of more precise gene-editing tools, accelerating the translation of CRISPR therapies from bench to bedside [30].

Path forward: from bench to bedside in autoimmune therapy

While CRISPR-based therapies hold significant promise for treating autoimmune diseases, several challenges remain. Ensuring the safety, efficacy, and ethical acceptability of these treatments is paramount. Ongoing research and clinical trials are essential to address these concerns and to refine gene-editing techniques for clinical application [31]. Collaborative efforts among scientists, clinicians, ethicists, and regulatory bodies will be crucial in navigating the complexities of CRISPR-based therapies. Establishing robust regulatory frameworks and ethical guidelines will facilitate the responsible integration of these innovative treatments into clinical practice, ultimately improving outcomes for patients with autoimmune diseases [32].

Conclusions

The integration of CRISPR-Cas technology into the field of immunology has opened transformative possibilities for the treatment of autoimmune diseases. This review highlighted the critical role of immune checkpoints, specifically CTLA-4 and PD-1, in maintaining immune tolerance and preventing aberrant autoimmune responses. Dysregulation of these checkpoints is a hallmark of several autoimmune conditions, and current therapies targeting them often suffer from lack of specificity, transient effects, and immune-related adverse events.

CRISPR-based gene editing offers a novel, precise, and durable approach to modulating these checkpoints. With advancements such as CRISPRa/i, prime editing, and epigenetic tools, researchers can now fine-tune gene expression without permanently altering the genome, allowing for reversible and safer therapeutic interventions. Moreover, the combination of CRISPR with nanotechnology and AI-driven design holds promise for enhancing specificity, reducing off-target effects, and accelerating clinical translation. Despite its promise, significant challenges remain—including delivery efficiency, immune-related safety, regulatory approval, and ethical concerns. Continued preclinical and clinical investigations are essential to bridge existing gaps. With careful oversight and innovation, CRISPR-mediated checkpoint editing could redefine the future of autoimmune disease therapy, providing patient-specific, long-lasting solutions where conventional therapies fall short.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Paluch C, Santos AM, Anzilotti C, Cornall RJ, Davis SJ. Immune checkpoints as therapeutic targets in autoimmunity. Front Immunol. 2018;9:2306. https://doi.org/10.3389/fimmu.2018.02306
2. Mohammadzadeh I, Qujeq D, Yousefi T, Ferns GA, Maniati M, Vaghari-Tabari M. CRISPR/Cas9 gene editing: A new therapeutic approach in the treatment of infection and autoimmunity. IUBMB life. 2020;72(8):1603-1621. https://doi.org/10.1002/iub.2296
3. Zhai Y, Moosavi R, Chen M. Immune checkpoints, a novel class of therapeutic targets for autoimmune diseases. Front Immunol. 2021;12:645699. https://doi.org/10.3389/fimmu.2021.645699
4. Huang C, Zhu HX, Yao Y, Bian ZH, Zheng YJ, Li L, et al. Immune checkpoint molecules. Possible future therapeutic implications in autoimmune diseases. J Autoimmun. 2019;104:102333. https://doi.org/10.1016/j.jaut.2019.102333
5. Lee MH, Shin JI, Yang JW, Lee KH, Cha DH, Hong JB, et al. Genome editing using CRISPR-Cas9 and autoimmune diseases: A comprehensive review. Int J Mol Sci. 2022;23(3):1337. https://doi.org/10.3390/ijms23031337

https://doi.org/10.61577/jiri.2025.100001

6. Wang K, Zhu Q, Lu Y, Lu H, Zhang F, Wang X, et al. CTLA-4+ 49 G/A polymorphism confers autoimmune disease risk: an updated meta-analysis. Genet Test Mol Bioma. 2017;21(4):222-227. https://doi.org/10.1089/gtmb.2016.0335
7. Tanhapour M, Vaisi-Raygani A, Khazaei M, Rahimi Z, Pourmotabbed T. Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) polymorphism, cancer, and autoimmune diseases. AIMS Medical Science. 2017;4(4):395-412. https://doi.org/10.3934/medsci.2017.4.395
8. Kassardjian A, Shintaku PI, Moatamed NA. Expression of immune checkpoint regulators, cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death-ligand 1 (PD-L1), in female breast carcinomas. PloS one. 2018;13(4):e0195958. https://doi.org/10.1371/journal.pone.0195958
9. Nosrat ZS, Nosrat ZM, Atighi S, Dianat T, Kord TK. Promoter methylation and expression status of cytotoxic T-lymphocyte-associated antigen-4 gene in patients with lupus. J Epigenet. 2021;2(1):31-38. https://doi.org/10.22111/jep.2020.30124.1019
10. Wang Y, Zhou S, Yang F, Qi X, Wang X, Guan X, et al. Treatment-related adverse events of PD-1 and PD-L1 inhibitors in clinical trials: a systematic review and meta-analysis. JAMA Oncol. 2019;5(7):1008-1019. https://doi.org/10.1001/jamaoncol.2019.0393
11. Yang H, Patel DJ. CasX: A new and small CRISPR gene-editing protein. Cell research. 2019;29(5):345-346. https://doi.org/10.1038/s41422-019-0165-4
12. Abdel-Wahab N, Shah M, Lopez-Olivo MA, Suarez-Almazor ME. Use of immune checkpoint inhibitors in the treatment of patients with cancer and preexisting autoimmune disease: a systematic review. Ann Intern Med. 2018;168(2):121-130. https://doi.org/10.7326/M17-2073
13. Barroso-Sousa R, Barry WT, Garrido-Castro AC, Hodi FS, Min L, Krop IE, et al. Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens: a systematic review and meta-analysis. JAMA Oncol. 2018;4(2):173-182. https://doi.org/10.1001/jamaoncol.2017.3064
14. Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. J Control Release. 2017;266:17-26. https://doi.org/10.1016/j.jconrel.2017.09.012
15. Singh V, Braddick D, Dhar PK. Exploring the potential of genome editing CRISPR-Cas9 technology. Gene. 2017;599:1-8. https://doi.org/10.1016/j.gene.2016.11.008
16. Wilbie D, Walther J, Mastrobattista E. Delivery aspects of CRISPR/Cas for in vivo genome editing. Acc Chem Res. 2019;52(6):1555-1564.
17. Sinclair F, Begum AA, Dai CC, Toth I, Moyle PM. Recent advances in the delivery and applications of nonviral CRISPR/Cas9 gene editing. Drug Deliv Transl Res. 2023;13(5):1500-1519.
18. Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. Nat Commun. 2018;9(1):3497. https://doi.org/10.1038/s41467-018-05843-9
19. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 2018;25(1): 1234-57. https://doi.org/10.1080/10717544.2018.1474964
20. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Sci. 2016;351(6268):84-88. https://doi.org/10.1126/science.aad5227
21. Kim S, Hupperetz C, Lim S, Kim CH. Genome editing of immune cells using CRISPR/Cas9. BMB Rep. 2021;54(1):59. https://doi.org/10.5483/BMBRep.2021.54.1.245
22. Goetzl EJ, Alpert JS. CRISPR-Cas gene editing to the genetic rescue. Am J Med. 2024;137(5):386-389. https://doi.org/10.1016/j.amjmed.2024.01.006
23. Khan SH. Genome-editing technologies: concept, pros, and cons of various genome-editing techniques and bioethical concerns for clinical application. Mol Ther Nucleic Acids. 2019;16:326-334. https://doi.org/10.1016/j.omtn.2019.02.027
24. Javid A, Numan M, Janiad S, Ahmed M. CRISPR: An Elixir for Autoimmune Diseases? A Systematic Review. LGU journal of life Sci. 2024;8(2):235-258. https://doi.org/10.54692/lgujls.2024.0802339
25. Boardman DA, Levings MK. Emerging strategies for treating autoimmune disorders with genetically modified Treg cells. J Allergy Clin Immunol. 2022;149(1):1-11. https://doi.org/10.1016/j.jaci.2021.11.007
26. Nicol D, Eckstein L, Morrison M, Sherkow JS, Otlowski M, Whitton T, et al. Key challenges in bringing CRISPR-mediated somatic cell therapy into the clinic. Genome Med. 2017;9:1-4. https://doi.org/10.1186/s13073-017-0475-4
27. Brokowski C, Adli M. CRISPR ethics: moral considerations for applications of a powerful tool. J Mol Biol. 2019;431(1):88-101. https://doi.org/10.1016/j.jmb.2018.05.044
28. Brokowski C, Adli M. Ethical considerations in therapeutic clinical trials involving novel human germline-editing technology. CRISPR J. 2020;3(1):18-26. https://doi.org/10.1089/crispr.2019.0051
29. Ernst MP, Broeders M, Herrero-Hernandez P, Oussoren E, van der Ploeg AT, Pijnappel WP. Ready for repair? Gene editing enters the clinic for the treatment of human disease. Mol Ther Methods clin dev. 2020;18:532-557. https://doi.org/10.1016/j.omtm.2020.06.022
30. Rees HA, Minella AC, Burnett CA, Komor AC, Gaudelli NM. CRISPR-derived genome editing therapies: Progress from bench to bedside. Mol Ther. 2021;29(11):3125-3139. https://doi.org/10.1016/j.ymthe.2021.09.027
31. Bhat AA, Nisar S, Mukherjee S, Saha N, Yarravarapu N, Lone SN, et al. Integration of CRISPR/Cas9 with artificial intelligence for improved cancer therapeutics. J Transl Med. 2022;20(1):534. https://doi.org/10.1186/s12967-022-03765-1
32. Uddin F, Rudin CM, Sen T. CRISPR gene therapy: applications, limitations, and implications for the future. Front oncol. 2020;10:1387. https://doi.org/10.3389/fonc.2020.01387

https://doi.org/10.61577/jiri.2025.100001