REVIEW

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Immunomodulation by Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-Associated Protein 9

Kümelenmiş Düzenli Aralıklı Kısa Palindromik Tekrarlar (CRISPR)/CRISPR ile İlişkili Protein 9 ile İmmünomodülasyon

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ABSTRACT

Inspired by a bacterial viral defense mechanism, Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) is a groundbreaking genome editing technology. It makes it possible for researchers to precisely alter DNA sequences in living things, enabling unheard-of precision and efficiency in gene editing. With the ability to create genetically modified creatures, treat genetic illnesses, and further scientific study, CRISPR-Cas9 holds great promise in the field of medicine. The term "immunomodulation" describes the control or modification of immune system function to produce targeted therapeutic effects. It involves modifying immune responses to reduce excessive inflammation, inhibit autoimmune reactions, or strengthen immunity against infections. Immune system functions are manipulated to accomplish the intended therapeutic results. Recent developments in the field of immunomodulation have shown promise in viral infections since immunomodulation has immediate applications in both scientific and clinical research. However, ethical issues need to be considered. In this review, we have discussed the process of the CRISPR/Cas9 technique and how this technique is adapted to the immunomodulation by each component of the immune system.

Keywords: CRISPR/Cas9, immunomodulation, immune system

ÖZ

Kümelenmiş Düzenli Aralıklı Kısa Palindromik Tekrarlar (CRISPR)/CRISPR ile ilişkili protein 9 (Cas9), bakteriyel bir viral savunma mekanizmasından ilham alınarak geliştirilmiş devrim niteliğinde bir genom düzenleme teknolojisidir. Bu teknoloji, araştırmacıların canlı organizmalardaki DNA dizilerini hassas bir şekilde değiştirmelerine olanak tanır ve gen düzenlemede daha önce görülmemiş bir kesinlik ve verimlilik sağlar. CRISPR-Cas9, genetik olarak değiştirilmiş canlılar oluşturma, genetik hastalıkları tedavi etme ve bilimsel çalışmaları ilerletme yeteneği ile tıpta büyük bir umut vaat etmektedir.

"İmmünomodülasyon" terimi, hedeflenen terapötik etkiler elde etmek amacıyla bağışıklık sistemi fonksiyonlarının kontrol edilmesi veya değiştirilmesi anlamına gelir. Aşırı iltihabı azaltmak, otoimmün reaksiyonları engellemek veya enfeksiyonlara karşı bağışıklığı güçlendirmek amacıyla bağışıklık yanıtlarının değiştirilmesini içerir. Bağışıklık sistemi işlevleri, istenen terapötik sonuçlara ulaşmak için manipüle edilir. İmmünomodülasyon alanındaki son gelişmeler, immünomodülasyonun hem bilimsel hem de klinik araştırmalarda doğrudan uygulamaları olduğu için, hayati enfeksiyonlar konusunda umut verici bir gelişme göstermiştir. Bununla birlikte, etik sorunlar da göz önünde bulundurulmalıdır. Bu derlemede, CRISPR/Cas9 tekniğinin sürecini ve bu tekniğin bağışıklık sisteminin her bir bileşeni tarafından immünomodülasyona nasıl adapte edildiğini tartıştık.

Anahtar Kelimeler: CRISPR/Cas9, immünomodülasyon, bağışıklık sistemi

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Introduction

Understanding Immunomodulation by Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-Associated Protein 9 (Cas9)

The CRISPR-Cas system, found in the majority of bacteria (47%) and archaea (87%), is an adaptive immune system that protects the cell against foreign genetic elements. CRISPR refers to clusters of regularly interspaced short palindromic repeates, and Cas refers to enzymes involved in the recognition and degradation of foreign DNA in the CRISPR system. This technology, widely used in scientific research areas, drastically changes the field of medicine. CRISPR/Cas9 technology, which ensures genome conservation and modulates gene function in cells and organisms, is cheaper, faster, easier to use and more convenient that previous gene editin technologies such as meganucleases, zinc finger nucleases and transcription activatior-like effector nucleases (1,2). For instance, this system can be modulated in almost any organism, even including human embryos (3). The CRISPR technique is currently being studied in many areas, especially for treating hemoglobinopathies, which is one of the approved applications of the *CRISPR* gene editing system. Blindness (4), mitochondrial diseases (5), genetic blood diseases (6), and lung diseases (7), are also examples of promising research areas of CRISPR. In addition, it has many applications in vital viral infections, including Coronavirus Disease 2019 and AIDS (8-10).

The mechanism of the CRISPR/Cas9 system includes a DNA-cutting element of bacterial immune systems that has been repurposed as a major tool for gene editing (Figure 1) (11). It functions as an exact pair of molecular scissors that can cut, modulate, and change a particular DNA sequence (12). The CRISPR/Cas9 system has two simple components: a guide RNA sequence (gRNA), which directs the Cas nuclease to its target, and a Cas nuclease, which binds and cleaves the targeted DNA sequence (8). Bacterial immune systems include Cas nuclease, which breaks the DNA sequence of incoming viruses, bacteriophages, rendering them inoperable (13). After its DNA-cleaving and modulating ability was found to have a biological mechanism, it was swiftly utilized as a genome editing technique (14).

Mechanism of the CRISPR/Cas9

Adaptive immune system of bacteria and archaea is based on the CRISPR system (15). Cas nucleases, which are a class of enzymes that are capable of binding to DNA sequences and causing double-strand breaks, function significantly in genetic engineering (15). A protospacer that is a segment of viral DNA is cut by a Cas nuclease when an archaea or bacteria is infected by a virus (16). This biological response can be kept in the bacterial genome as an immunological memory using the fragments of viruses that are encountered by the bacterial cells of the bacterium (16). The placement of these fragments occurs between the repeated palindromic sequences, and that placement gives rise to the name CRISPR (16).

The bacteria can identify and eliminate the same virus after reinfection via Cas9 (17). CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) are vital requirements of Cas9 activation (18). Together with the tracrRNA acting as a scaffold, the complementary crRNA matches the viral spacer that was retained during the initial infection (19). Together they create a complex known as a gRNA. A brief region located downstream of the target site that is known as the protospacer adjacent motif (PAM) is checked by the Cas9 enzyme before the cutting stage (20). A double-stranded break (DSB) is produced by Cas9 when it detects a target in the PAM while searching the region upstream (21). Due to the lack of inherent DNA repair systems of viruses, DSBs render the virus inoperable (22). CRISPR/Cas9 and recombinant DNA technology are both essential tools for gene editing technologies. However, they have some differences, as shown in Table 1.

Jinek et al. (23) discovered that the CRISPR-Cas9 system of bacteria and archaea, which can modulate any desired position in any genome of organisms, in 2012.

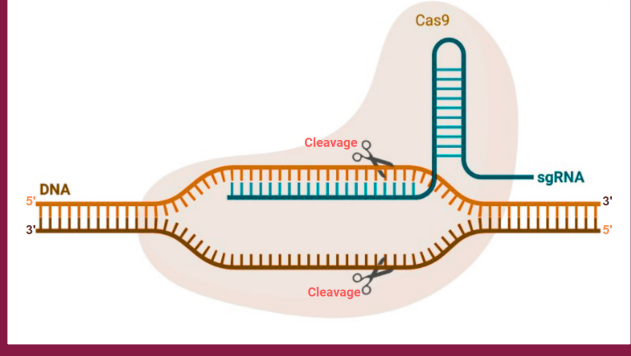

Figure 1. CRISPR/Cas9 system. The diagram illustrates the CRISPR/Cas9 system, a gene-editing tool that enables precise DNA modification. The single-guide RNA directs the Cas9 enzyme to a specific target sequence in the DNA by complementary base pairing
CRISPR: Immunomodulation by Clustered Regulatory Interspaced Short Palindromic Repeats, Cas9: CRISPR-associated protein 9

Table 1. Differences between recombinant DNA and CRISPR/Cas9

Differences	Recombinant DNA	CRISPR/Cas9
Process	By combining DNA from different sources	By using a guide RNA
Enzymes	Ligase	Cas9
Applying	By inserting randomly into the host genome	By editing in specific locations in the host genome
Precision	Low	High
Non-rarget effects	High risk	Low risk
Complexity	More complex	Simple
Ethical regulation	Well-established	Concerns

CRISPR: Immunomodulation by Clustered Regulatory Interspaced Short Palindromic Repeats, Cas9: CRISPR-associated protein 9

Modulation of the Immune System by CRISPR-Cas9

Many diseases that affect people are caused by viral infections, including the deadly Acquired Immunodeficiency Syndrome (AIDS). Cures for these crucial illnesses currently center on medications and vaccinations that either directly target the vital proteins or prevent the virus from interfering with the host cells (24). However, viral infections like AIDS occur by the virus that enters the host immune cells and merges with the host genome to form an infection (25). This poses a critical challenge to the development of effective vaccinations. The main obstacle to developing effective cures is the inability of the immune system to eradicate the viral DNA from reservoirs (26).

Accurate CRISPR/Cas9-based modification of immune system cells for therapeutic activity is currently regarded as a groundbreaking development in immunotherapeutic strategies to prevent cancer spread. Expression of CRISPR/Cas9 can be established by viral vectors such as adenovirus, lentivirus, and nanomaterials such as graphene, zeolite imidazole, and cell penetrating protein (27).

Modulation of Immune System Cells by CRISPR/Cas9

T-cells are essential to the adaptive immune system. They are necessary for identifying and reacting to infections, cancer cells, and pathogens. Through the introduction of specific modifications, CRISPR/Cas9 allows for precise manipulation of the T-cell genome. In cytokines such as interleukin 2, interleukin 4, and interferon gamma, manipulation of T-cells can be performed by gene insertions, gene deletions, and gene alterations to target infectious cells. Also, owing to CRISPR/Cas9 technology, chimeric antigen receptor T-cell therapy can be performed against the tumor antigens (28).

An essential component of the innate immune response is natural killer (NK) cells, a subset of lymphocytes in the immune system. NK cells can identify and react to malignant or infected cells without the need for prior sensitization. They are also members of the adaptive immune system and depend on having previously been exposed to a particular antigen in order to operate properly. To effectively utilize NK cell modulation, certain pathways may need to be suppressed while activating others since NK cell immunity has multiple pathways. Manipulation of NK cells can be performed in the presence of specific cytokines such as interleukin 2, interleukin 15, interleukin 18, (29,30). In addition, upregulation of tumour-specific chemokine receptor can be established by NK cell immunotherapy (31).

One antigen-presenting cell that is essential to the immune system is the dendritic cell (DC). By delivering antigens to T-cells, they serve as intermediaries between the innate and adaptive immune systems, triggering and modulating the adaptive immune response. Effectively manipulating human DCs is a difficult endeavor, despite reports of gene repression techniques using RNA interference. The majority of research on DC biology has been done on mice (32,33), yet adoptive and innate immunity are different in humans versus mice. In order to overcome all of these restrictions, a targeted knockout with a median efficiency of >94% across >300 genes was created directly in human monocyte-derived dendritic cells (moDCs) using the CRISPR/Cas9 technique. Using this technique, a genetic screen was conducted in moDCs to identify potential mechanisms by which DCs modify their response to lipopolysaccharides from the human microbiome (34).

B-cells play a crucial role in the adaptive immune system, primarily by producing antibodies that recognize and neutralize harmful substances such as bacteria, viruses, and toxins. CRISPR/Cas9 technology can be used to knock out specific genes in B-cells to investigate their roles in immune regulation, signaling pathways, and antibody production. For example, targeting genes such as CD19 and CD20 provides insights into their functions in B-cell activation and immune responses. Additionally, disrupting B-cell receptor (BCR) genes using CRISPR/Cas9 results in B-cells lacking functional BCR expression. This approach is considered advantageous for studying BCR signaling

and developing B-cell therapies where regulation of BCR signaling is required (35).

Immune Tolerance by CRISPR/Cas9

The term "immune tolerance" describes the immune system's lack of sensitivity or response to some antigens, such as innocuous environmental compounds and self-antigens. It is a vital component of immune control that keeps the immune system from developing excessive or dangerous reactions to harmless substances (allergies) and from attacking the body's own tissues (autoimmunity).

Targeting the underlying immunological dysregulation, CRISPR/Cas9-mediated immune tolerance presents a promising treatment option for autoimmune disorders (36). When compared to conventional treatments like immunosuppression, this may result in more targeted, efficient therapy with fewer adverse effects. Since the immune system frequently builds defenses against therapeutic gene products, immunological tolerance induction is critical to the success of gene therapy (37). The safety and effectiveness of gene therapy treatments could be increased by using CRISPR/Cas9 to modify immune cells so they can accept gene therapy vectors. However, there is a chance that long-term, systematic blocking antibody delivery will undermine immune tolerance and result in an immunological attack on healthy tissues (38).

Ethical Issues of Immunomodulation by CRISPR/Cas9

Ensuring the safety of individuals receiving immunomodulation therapy is a vital ethical concern. Although it is promising, CRISPR-Cas9 technology is still in its infancy, and before it is widely used in clinical applications,the hazards of off-target effects and unintended consequences must be fully recognized and reduced (39). It is imperative to guarantee fair access to immunomodulation medicines based on CRISPR. Limitations in access owing to variables like location, insurance coverage, or socioeconomic level could raise questions (40). To guarantee that everyone who potentially benefits from innovative treatments has access to them, efforts should be undertaken to alleviate these inequities. Immunomodulation mediated by CRISPR-Cas9 may have unforeseen effects on specific patients as well as larger social impacts (36). These might include unanticipated side effects,inadvertent changes to the human gene pool should germline editing be attempted, or the rise of disease strains with increased resistance. To evaluate the effectiveness and safety of CRISPR-Cas9-mediated immunomodulation over time, patients must be monitored for an extended period. Ensuring the proper systems are in place for continuous monitoring and assessment is part of the ethical considerations (41).

Challenges and Perspectives

CRISPR-Cas9-based immunomodulation offers a promising new approach to treating a variety of diseases, but it also has its own set of difficulties and challenges. Unintentional genome editing using CRISPR-Cas9 technologies can occasionally have off-target consequences (36). This might lead to unforeseen immunological reactions or abnormalities in immune function in the setting of immunomodulation. In addition, immune response could be triggered after the introduction of CRISPR components or stages into the body, and potential inflammation or even rejection might occur (42). Furthermore, delivering CRISPR/Cas9 components to any specific immune cells is another significant challenge. It is necessary to create effective delivery strategies to guarantee that the intended modifications are implemented in the right cells without damaging neighboring tissues. It is still unclear exactly what CRISPR-mediated immunomodulation will adapt in the long term. It is imperative to take into account the possibility of unknown or unintentional impact in the long term (43).

With the use of CRISPR-Cas9 technology, precise immune system changes may be possible, enabling individualized patient-specific therapy. Furthermore, through the use of CRISPR-Cas9 for immunomodulation, vital autoimmune diseases may be treated by focusing on immune cells or pathways that are particular to the disease process (44,45). Because CRISPR-Cas9 technology offers a potent tool for examining gene activity and regulation in immune cells, it has already completely changed biomedical research. Further investigation in this field may yield fresh perspectives on the operation of the immune system and innovative treatment modalities (46).

Conclusion

CRISPR-Cas9 technology has revolutionized the field of genome editing, offering precise and efficient tools for modifying immune system components. By enabling targeted gene modifications, CRISPR technology has opened new avenues for understanding immune regulation, disease mechanisms, and potential therapeutic applications. The ability to manipulate T-cells, B-cells, NK cells, and DCs provides valuable insights into immunological functions and paves the way for novel treatments for infectious diseases, autoimmune disorders, and cancers. Additionally, CRISPR-Cas9-mediated immune tolerance presents a promising strategy for addressing autoimmune conditions while minimizing adverse effects compared to conventional therapies.

Despite its immense potential, challenges remain in addressing the safety, specificity, and ethical implications of CRISPR-based immunomodulation. Off-target effects, immune responses to CRISPR components, and long-term consequences of genetic modifications must be carefully assessed. Furthermore, equitable access to CRISPR-based therapies and regulatory considerations require ongoing attention to ensure responsible implementation in clinical settings.

Future research will continue to refine CRISPR-Cas9 applications in immunotherapy, enhance delivery systems, and address ethical concerns. With advancements in precision genome editing, this technology holds great promise for personalized medicine, improved immune system therapies, and a deeper understanding of immunological processes. Continued interdisciplinary collaboration will be essential in harnessing the full potential of CRISPR-Cas9 while ensuring its safe and ethical use in medical applications.

Ethics

Informed Consent: This study did not require informed consent as it did not involve human participants, animal subjects, or identifiable personal data.

Footnotes

Authorship Contributions

Data Collection or Processing: D.K., H.M.Ö., S.K.T., Literature Search: D.K., H.M.Ö., S.K.T., Writing: D.K., H.M.Ö., S.K.T.

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REFERENCES

1. Yun Y, Ha Y. *CRISPR/Cas9-mediated* gene correction to understand ALS. Int J Mol Sci. 2020;21:3801. [Crossref]
2. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther. 2020;5:1. [Crossref]
3. Görücü Yilmaz S. Genome editing technologies: CRISPR, LEAPER, RESTORE, ARCUT, SATI, and RESCUE. EXCLI J. 2021;20:19-45. [Crossref]
4. Ahmad I. CRISPR/Cas9-a promising therapeutic tool to cure blindness: current scenario and future prospects. Int J Mol Sci. 2022;23:11482. [Crossref]
5. Tang JX, Pyle A, Taylor RW, Oláhová M. Interrogating mitochondrial biology and disease using *CRISPR/Cas9* gene editing. Genes. 2021;12:1604. [Crossref]
6. Kohn Donald B. Gene therapy for blood diseases. Curr Opin Biotechnol. 2019;60:39-45. [Crossref]
7. Carneiro SP, Greco A, Chiesa E, Genta I, Merkel OM. Shaping the future from the small scale: dry powder inhalation of CRISPR-Cas9 lipid nanoparticles for the treatment of lung diseases. Expert Opin Drug Deliv. 2023;20:471-487. [Crossref]
8. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics. 2021;15:353-361. [Crossref]
9. Qin Y, Li S, Li XJ, Yang S. CRISPR-based genome-editing tools for Huntington's disease research and therapy. Neurosci Bull. 2022;38:1397-1408. [Crossref]
10. Li S, Huang J, Ren L, Jiang W, Wang M, Zhuang L, et al. A one-step, one-pot CRISPR nucleic acid detection platform (CRISPR-top): Application for the diagnosis of COVID-19. Talanta. 2021;233:122591. [Crossref]
11. Kick L, Kirchner M, Schneider S. CRISPR-Cas9: From a bacterial immune system to genome-edited human cells in clinical trials. Bioengineered. 2017;8:280-286. [Crossref]
12. Sharma AK, Giri AK. Engineering CRISPR/Cas9 therapeutics for cancer precision medicine. Front Genet. 2024;15:1309175. [Crossref]
13. Leon LM, Mendoza SD, Bondy-Denomy J. How bacteria control the CRISPR-Cas arsenal. Curr Opin Microbiol. 2018;42:87-95. [Crossref]
14. Du Y, Liu Y, Hu J, Peng X, Liu Z. CRISPR/Cas9 systems: Delivery technologies and biomedical applications. Asian J Pharm Sci. 2023;18:100854. [Crossref]
15. Newsom S, Parameshwaran HP, Martin L, Rajan R. The CRISPR-Cas mechanism for adaptive immunity and alternate bacterial functions fuels diverse biotechnologies. Front Cell Infect Microbiol. 2021;10:619763. [Crossref]
16. Zhang D, Zhang Z, Unver T, Zhang B. CRISPR/Cas: A powerful tool for gene function study and crop improvement. J Adv Res. 2020;29:207-221. [Crossref]
17. Hille F, Charpentier E. CRISPR-Cas: biology, mechanisms and relevance. Philos Trans R Soc Lond B Biol Sci. 2016;371:20150496. [Crossref]
18. Le Rhun A, Escalera-Maurer A, Bratovič M, Charpentier E. CRISPR-Cas in *Streptococcus pyogenes*. RNA Biol. 2019;16:380-389. [Crossref]
19. Liu Y, Liu W, Wang B. Engineering CRISPR guide RNAs for programmable RNA sensors. Biochem Soc Trans. 2023;51:2061-2070. [Crossref]
20. Liao C, Beisel CL. The tracrRNA in CRISPR biology and technologies. Annu Rev Genet. 2021;55:161-181. [Crossref]
21. Shumega AR, Pavlov YI, Chirinskaite AV, Rubel AA, Inge-Vechtomov SG, Stepchenkova EI. CRISPR/Cas9 as a mutagenic factor. Int J Mol Sci. 2024;25:823. [Crossref]
22. Huang RX, Zhou PK. DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer. Signal Transduct Target Ther. 2020;5:60. [Crossref]
23. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337:816-821. [Crossref]
24. Burrell CJ, Howard CR, Murphy FA. Pathogenesis of virus infections. Fenner and White's Medical Virology. 2016;77-104. [Crossref]
25. Faivre N, Verollet C, Dumas F. The chemokine receptor CCR5: multi-faceted hook for HIV-1. Retrovirology. 2024;21:2. [Crossref]
26. Zhang G, Tang T, Chen Y, Huang X, Liang T. mRNA vaccines in disease prevention and treatment. Signal Transduct Target Ther. 2023;8:365. [Crossref]
27. Ou X, Ma Q, Yin W, Ma X, He Z. *CRISPR/Cas9* gene-editing in cancer immunotherapy: promoting the present revolution in cancer therapy and exploring more. Front Cell Dev Biol. 2021;9:674467. [Crossref]
28. Park H, Kang YK, Shim G. CRISPR/Cas9-mediated customizing strategies for adoptive T-cell therapy. Pharmaceutics. 2024;16:346. [Crossref]
29. Qi YY, Lu C, Ju Y, Wang ZE, Li YT, Shen YJ, et al. Interleukin-18 synergism with interleukin-2 in cytotoxicity and NKG2D expression of human natural killer cells. Asian Pacific Journal of Cancer Prev. 2014;18:7857-7861. [Crossref]
30. Szczepanski MJ, Szajnik M, Welsh A, Foon KA, Whiteside TL, Boyiadzis M. Interleukin-15 enhances natural killer cell cytotoxicity in patients with acute myeloid leukemia by upregulating the activating NK cell receptors. Cancer Immunol Immunother. 2010;59:73-79. [Crossref]

31. Freund-Brown J, Chirino L, Kambayashi T. Strategies to enhance NK cell function for the treatment of tumors and infections. Crit Rev Immunol. 2018;38:105-130. [Crossref]
32. Kim B, Lee YE, Yeon JW, Go GY, Byun J, Lee K, et al. A novel therapeutic modality using CRISPR-engineered dendritic cells to treat allergies. Biomaterials. 2021;273: 120798. [Crossref]
33. Jost M, Jacobson AN, Hussmann JA, Cirolia G, Fischbach MA, Weissman JS. CRISPR-based functional genomics in human dendritic cells. Elife. 2021;10:65856. [Crossref]
34. Maschalidi S, Mehrotra P, Keçeli BN, De Cleene HKL, Lecomte K, Van der Cruyssen R, et al. Targeting SLC7A11 improves efferocytosis by dendritic cells and wound healing in diabetes. Nature. 2022;606:776-784. [Crossref]
35. Allemailem KS, Alsahli MA, Almatroudi A, Alrumaihi F, Al Abdulmonem W, Moawad AA, et al. Innovative strategies of reprogramming immune system cells by targeting CRISPR/Cas9-based genome-editing tools: a new era of cancer management. Int J Nanomedicine. 2023;18:5531-5559. [Crossref]
36. Rasul MF, Hussen BM, Salihi A, Ismael BS, Jalal PJ, Zanichelli A, et al. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. Mol Cancer. 2022;21:64. [Crossref]
37. Freitas MV, Frâncio L, Haleva L, Matte UDS. Protection is not always a good thing: The immune system's impact on gene therapy. Genet Mol Biol. 2022;45:20220046. [Crossref]
38. Abdelnour SA, Xie L, Hassanin AA, Zuo E, Lu Y. The Potential of *CRISPR/Cas9* gene editing as a treatment strategy for inherited diseases. Front Cell Dev Biol. 2021;9:699597. [Crossref]
39. Shinwari ZK, Tanveer F, Khalil AT. Ethical issues regarding CRISPR mediated genome editing. Curr Issues Mol Biol. 2018;26:103-110. [Crossref]
40. Subica AM. CRISPR in Public Health: The health equity implications and role of community in gene-editing research and applications. Am J Public Health. 2023;113:874-882. [Crossref]
41. Rubeis G, Steger F. Risks and benefits of human germline genome editing: An ethical analysis. Asian Bioeth Rev. 2018;10:133-141. [Crossref]
42. Liu W, Li L, Jiang J, Wu M, Lin P. Applications and challenges of *CRISPR-Cas* gene-editing to disease treatment in clinics. Precis Clin Med. 2021;4:179-191. [Crossref]
43. 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. [Crossref]
44. 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;23:1337. [Crossref]
45. Roth-Walter F, Adcock IM, Benito-Villalvilla C, Bianchini R, Bjermer L, Boyman O, et al. Immune modulation via T regulatory cell enhancement: Disease-modifying therapies for autoimmunity and their potential for chronic allergic and inflammatory diseases-An EAACI position paper of the Task Force on Immunopharmacology (TIPCO). Allergy. 2021;76:90-113. [Crossref]
46. Chen X, Zhong S, Zhan Y, Zhang X. CRISPR-Cas9 applications in T cells and adoptive T cell therapies. Cell Mol Biol Lett. 2024;29:52. [Crossref]