The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 7, 2025

SYNERGIZING CRISPR-CAS9 GENE EDITING WITH PHARMACOLOGICAL INTERVENTIONS: EMERGING PARADIGMS IN GENETIC DISORDER THERAPEUTICS

Mehwish Nisar[1], Zufi Shad[2], Sidra Siddiqui*[3], Iqra Haider[4], Tahmina Maqbool[5]

[1,2,*3,4,5]*Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Madinat al-Hikmah, Hakim Mohammed Said Road, Karachi – 74600, Pakistan.*

[*3]Sidra.siddiqui@hamdard.edu.pk

DOI: https://doi.org/10.5281/zenodo.16018105

Keywords
CRISPR-based gene therapy; Pharmacological treatments; Genetic disorders; Gene editing; Precision medicine; CRISPR technology; Combination approach

Article History
Received: 11 April, 2025
Accepted: 02 July, 2025
Published: 17 July, 2025

Corresponding Author: * Sidra Siddiqui

Abstract
The combination of CRISPR-based gene editing and pharmacological therapies offers a promising strategy for treating genetic diseases. CRISPR enables precise genome modifications (base/prime editing, knock-in/out), while advanced delivery systems enhance its therapeutic potential. In cancer immunotherapy, CRISPR-engineered CAR-T cells address challenges like autologous production, drug resistance, and toxicity. Combining CRISPR with drugs improves efficacy, overcomes resistance, and optimizes delivery by targeting DNA repair pathways. However, challenges such as off-target effects, immune responses, and ethical concerns remain. Pharmacological agents can boost CRISPR precision by inhibiting DNA repair. Future efforts should refine CRISPR systems, integrate AI-driven personalized medicine, and tackle polygenic diseases. Rigorous research, ethical oversight, and regulatory frameworks are essential before clinical adoption. While progress is encouraging, further improvements in safety, efficacy, and accessibility are needed to establish this approach as a mainstream therapy.

INTRODUCTION

BACKGROUND

Gene therapy, particularly Adeno-associated viruses AAV vectors, offers promising root-cause treatment for 7,000+ genetic diseases, though challenges in complexity and ethics persist amid ongoing clinical progress (1). The goal of gene therapy is to slow the progression of neurodevelopmental disorders by developing adeno-associated viral vectors that target diseases of the central nervous system, potentially revolutionizing treatment options(2). Ex vivo gene editing is a technique used to treat hereditary skin disorders by repairing defective genes in a patient's external cells and reintroducing them into the body (3). Luxturna®, the first approved gene therapy for Leber congenital amaurosis type, 2 is one of several clinical trials being conducted to assess gene therapies for inherited retinal diseases (IRDs) (4). Another innovative approach is prime editing, which enables precise genetic alterations, including insertions, deletions, and base changes. This technique has been investigated in preclinical studies for conditions like cystic fibrosis, beta-thalassemia, and neurodegenerative diseases (5).

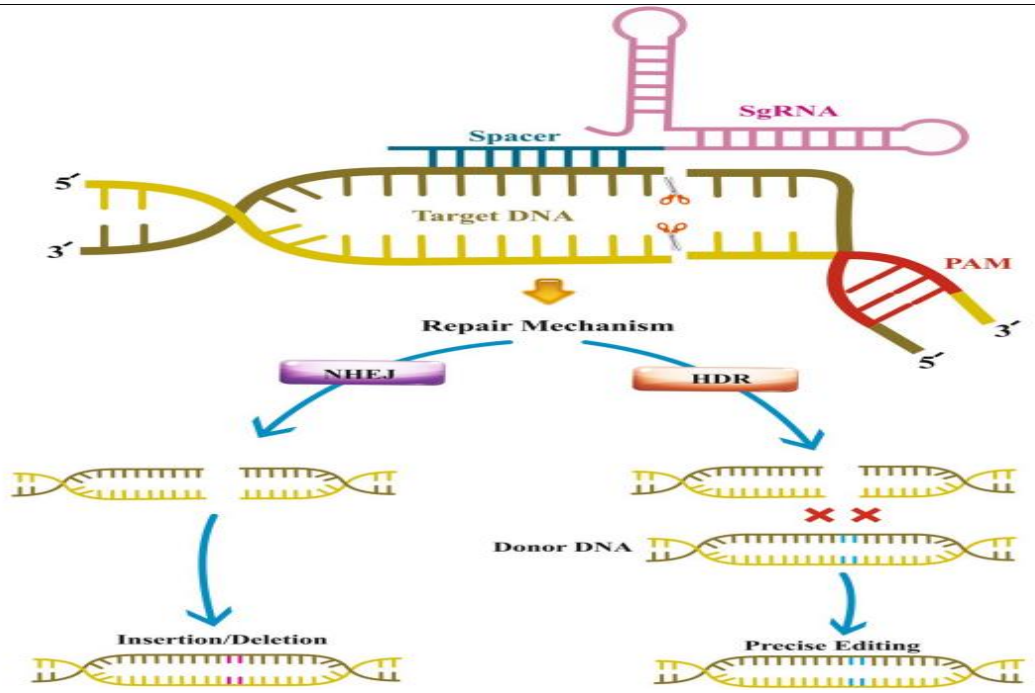
EMERGENCE OF GENETIC THERAPY

Targeting oncogenic pathways with high-quality treatment may be a promising cancer treatment.

This strategy has been improved by developments in molecular biology, such as programmable nucleases like ZFNs, TALENs, and CRISPR/Cas9 systems, which enable accurate targeting and change of genetic information within cancer cells, potentially providing therapeutic advantages (6). By using nanoparticles, which can be either inorganic or biological, nanomedicine is improving the delivery and effectiveness of gene therapy for the treatment of cancer (7). Although they encounter difficulties in clinical application, nonviral gene therapy vectors such as gene transfer, RNA interference, and epigenetic regulation offer promise in the treatment of breast cancer (8). Similarly, gene therapy for gastrointestinal cancers involves the use of therapeutic genes and the exploration of vectors, with recent trials showing encouraging safety and efficacy profiles, despite not being widely adopted in clinical practice yet. (9). By modifying cancer-causing mutations, boosting immune responses, and upsetting tumor survival systems, gene therapy, immune-based therapies, and CRISPR technologies are transforming precision medicine(10). Thanks to continuing clinical trials and improvements in delivery technologies, gene therapy has a lot of promise for personalized cancer treatments(11).

INTRODUCTION TO CRISPR-CAS SYSTEMS

CRISPR-based gene therapy combined with pharmacological treatments holds promise for genetic diseases, but requires advanced delivery systems and rigorous clinical validation for safe, effective translation(12). Gene-editing technology provides therapeutic options for hereditary illnesses such as cystic fibrosis, thalassemia, and Duchenne muscular dystrophy by precisely targeting particular genes to create desirable modifications (13). While CRISPR offers therapeutic potential, challenges like off-target effects and delivery efficiency must be resolved for clinical success (14). Combining CRISPR/Cas9-based genome editing with drug therapies holds the potential to enhance treatment efficacy. For instance, CRISPR can be used to make genetic edits that bolster the body's response to drugs or reduce resistance, thereby amplifying drug effectiveness (15). In cancer therapy, integrating CRISPR technology with existing chemotherapeutic regimens could improve outcomes by targeting and modifying genes associated with drug resistance or by making cancer cells more susceptible to treatment (16). This interdisciplinary approach could eventually lead to breakthrough treatments for genetic disorders, offering patients novel and potentially curative options (17) as illustrated in Fig. no 1.



The mechanisms of action of the CRISPR/Cas9 system, including nonhomologous end-joining (NHEJ), homology-directed repair (HDR), single-guide RNA (sgRNA), and protospacer adjacent motif (PAM),(18)

RATIONALE FOR COMBINATION THERAPIES

The rationale for employing combination therapies in medicine is multi-faceted, with the goal of enhancing treatment outcomes, reducing side effects, and overcoming drug resistance.

1.Enhanced Efficacy and Synergy: Combination therapies are designed to target multiple pathways simultaneously, providing a more effective response than monotherapy alone. For instance, in cancer treatment, combining different drugs can inhibit several cancer-signaling pathways or functions, thereby maximizing therapeutic impact (19). This approach has been particularly successful in oncology and is applied in other areas like obesity treatment and multiple sclerosis(20).

2. Reduced Toxicity: Combining drugs with lower doses minimizes toxicity, especially in cancer treatments, where achieving optimal therapeutic doses without severe side effects is challenging(21).

3. Overcoming Drug Resistance: Combination therapies targeting multiple disease progression pathways, such as DNA repair inhibition in cancer cells, can enhance treatment efficacy against resistant cancer cells, a significant challenge in disease management(22).

4.Personalized and Precision Medicine: Advances in systems pharmacology and computational modeling enable personalized combination therapies, improving patient outcomes and minimizing side effects by understanding disease mechanisms and drug interactions (23).

5. Multimodality Strategies: Multimodal therapies improve outcomes, minimize resistance, and tailor treatment for aggressive cancers(24).

CRISPR-BASED GENE THERAPY

RNA-guided Cas proteins enable precise targeting, while miniaturized CRISPR systems expand gene-

editing potential by easing delivery challenges (25). CRISPR-Cas uses spacer-containing arrays to direct Cas proteins in identifying and cutting specific nucleic acid targets.(26) CRISPR-Cas combines spacer integration and DNA targeting for immunity, with evolutionary roots in mobile elements enabling genome-editing applications. (27) CRISPR-Cas systems face challenges like off-target effects and Cas toxicity, but strategies include developing novel variants with improved specificity and tailored delivery methods (28). Lastly, despite the extensive applications in gene editing, CRISPR-Cas systems face regulatory and functional complexities that require further exploration to enhance their efficacy and expand their technological repertoire (29).

Types Of Genetic Modifications: Genetic engineering techniques like base editing, prime editing, knock-out, and knock-in have revolutionized gene function study, disease modeling, and personalized treatments, particularly in bone marrow cancers like leukemia and multiple myeloma.

1)Knock-Out And Knock-In Modifications: CRISPR/Cas9 system is utilized for knock-out and knock-in genetic modifications, causing double-stranded breaks at specific loci, often introducing frameshift mutations through non-homologous end joining pathways.(30) Knock-in involves introducing a foreign DNA sequence into a specific locus, facilitated by HDR pathways, for in-depth functional genetics studies and therapeutic applications like animal disease models (31).

2)Base Editing: Base editing is an advancement that allows precise conversion of one nucleotide base pair into another without creating double-strand breaks, thus reducing the risk of introducing unwanted insertions or deletions (32). Two main types of base editors have been developed—cytosine base editors (CBEs) and adenine base editors (ABEs), which allow for C-to-T and A-to-G conversions, respectively. These editors have shown potential in correcting point mutations responsible for genetic disorders by altering single nucleotide polymorphisms (33).

3)Prime Editing: Prime editing is a highly versatile genome-editing technique that enables precise editing without the requirement of donor DNA or double-strand breaks. It can perform a wider array of edits, including all 12 possible base-to-base conversions, insertions, and deletions, making it an innovative tool for correcting a wider range of genetic mutations (34).

APPLICATIONS AND INTEGRATION

These genetic modification tools are being rapidly integrated into both basic and applied research settings, including plant biology, microbial engineering, and therapeutic gene editing.(35) They offer potential treatment strategies for genetic diseases and hold promise for developing improved crop varieties and novel cell lines for industrial applications (36) as shown in table no. 1

Table 1. Utilizing CRISPR/Cas9 to treat infectious diseases

Virus type	Target gene	Cell/animal	Delivery method	Result	Ref
HPV-16	E7	SiHa, Caski, C33A, and HEK293 cell lines	Plasmid	Induction of apoptosis and inhibition of tumor cell growth	(37)
HPV-16	E7	Mice	PEGylated liposome	Elimination of established tumors in immunocompetent mice	(38)

HIV-1	LTR	Jurkat cells and HeLa cell line	Plasmid	Efcient cleavage of LTR target sites	(39)
HPV-16	E6, E7	Mice	Plasmid	Activation of p53 and pRB signaling pathways, leading to impaired tumor growth	(40)
HBV	Various sites	Huh-7 cell line Mice	Plasmid	Clearance of intrahepatic HBV templates in vivo	(41)
HIV-1	LTR U3, T, and R region	HEK293T cell line	Lentivirus	Enabling prolonged adaptive defense versus new viral infection	(42)
HIV-1	CXCR4	Ghost-CXCR4 cells, Jurkat cells, and primary human CD4+ T cells	Lentivirus	Resistance to HIV infection	(43)
HPV-16	E6/E7	SiHa cell line	Lipofectamine	Synergistic antitumor efect of E6/E7 KO using CRISPR system with PD1 inhibitors of cancer cell	(44)
HPV-18	E7	Hela cell line Mice	Micelle delivery, Lipofectamine	Reducing the HPV induced cancerous activity	(45)
HIV-1	LTR	HEK293T TZM-bl cells	Plasmid	Suppressing HIV-1 replication	(46)

CRISPR/CAS9 IN CAR-T CELL THERAPIES

T effector cells have been genetically engineered using chimeric antigen receptors (CARs) to enhance tumoricidal and adoptive cellular therapy (ACT) effects (47). CARs, or recombinant synthetic surface receptors, identify cancer cell antigens and activate redirected effector cells, with a single-chain variable being the fundamental construct (48, 49). Among the hematological malignancies for which CAR-T cell therapy has shown exceptional results are multiple myeloma (MM), lymphoma, acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL),(50) CAR-T cell research and development has also shown great promise in solid tumors like non-small cell lung cancer, melanoma, breast cancer, and sarcoma, (51, 52) CAR-T cell therapy faces three major barriers: individual autologous cell generation, cancer cell resistance, and undesirable toxicities and cytokine release syndrome. (CRS) Autologous CAR-T cells must be made on an individual basis, which hinders their widespread clinical use because of the costly and time-consuming production process. (53-55) In response to similar ligands produced by cancer cells, induced CAR-T cells may express immunological checkpoint molecules such as PD1, lymphocyte activation gene 3 (LAG3), or CD223. This inhibits CAR-T cells' ability to fight cancer.

(56, 57) **Fig 2** CRS may be brought on by increased GM-CSF, IL-6, and IL-1 release in addition to the concurrent activation of a sizable number of CAR-T cells (58), as illustrated in figure number 2.

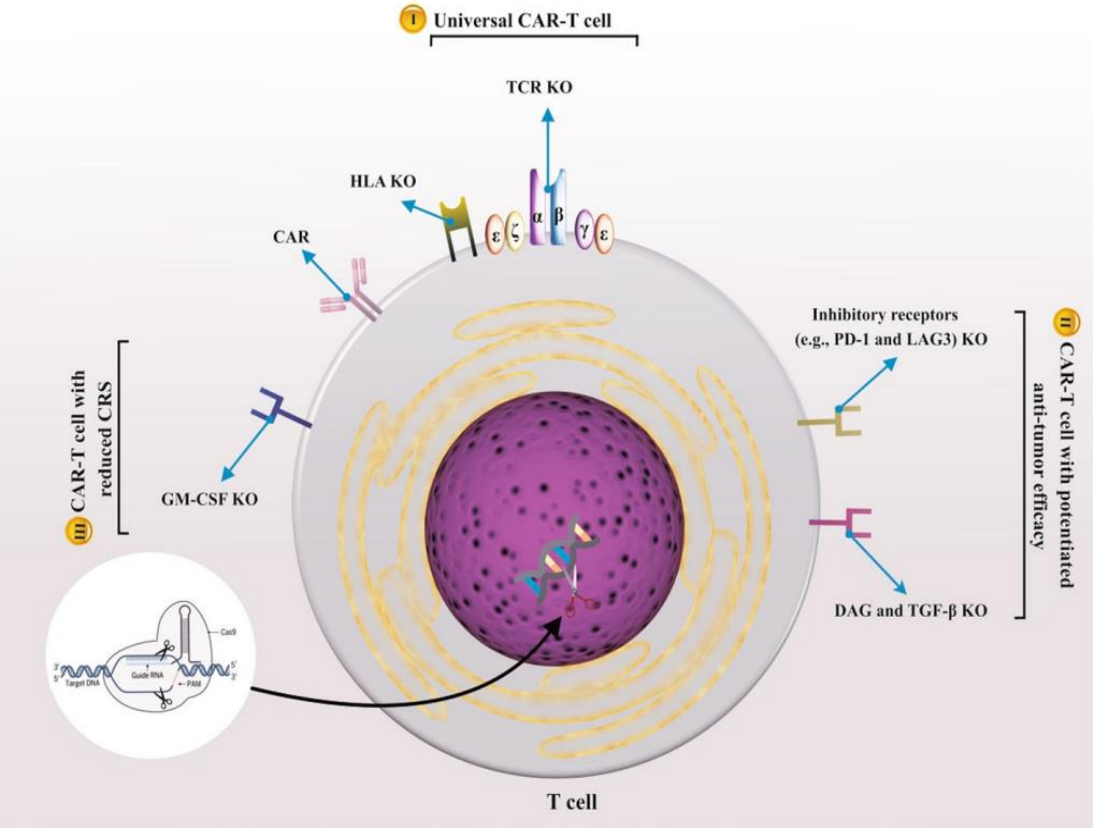


Fig 2: Utilizing a range of genes, including KO, HLA, GM-CSF, TCR, LAG-3, TGF-βR, DAG, and PD1, CRISPR/Cas9 enables future CAR-T cell production.(18)

DELIVERY METHODS

Gene and drug delivery requires both viral and non-viral delivery methods; viral vectors, such as AAVs, are very effective for precise gene editing in vivo**(59).** However, concerns such as immunogenicity and potential for mutagenesis have shifted attention towards non-viral vectors (60). Nanoparticles, a non-viral vector, offer safer, targeted delivery of therapeutic agents, improving effects and overcoming limitations like poor water solubility and passive targeting (61). Because of its versatility, electroporation a physical technique that briefly permeabilizes cell membranes using electric pulses is employed in gene electro transfer and therapeutic applications (62). The use of nanoparticles as drug carriers is further characterized by their ability to target specific sites within the body, utilizing passive and active targeting strategies, enhancing pharmacokinetics and diminishing systemic toxicity (63). The development of biomimetic nanoparticles, which mimic natural biological carriers, exemplifies the innovation in overcoming biological delivery barriers (64), While each delivery method presents unique benefits, they also confront limitations. Viral vectors still grapple with safety concerns, whereas non-viral strategies like nanotechnology must continue to address delivery efficiency and specificity (65). Continuous advancements in these technologies, guided by a growing understanding of their mechanisms and

applications, are pivotal in optimizing their roles in therapeutic contexts (66).

Limitations And Challenges

Gene therapy treats hereditary and acquired diseases by using genome-editing tools such as Crispr-cas9. These therapies may, however, have unanticipated side effects, immunological responses, and mutations. To guarantee the effectiveness and safety of gene-based therapies, meticulous planning, testing, and creativity are essential.

Off-Target Effects: Despite advances in technology, off-target consequences from CRISPR-based gene editing can result in detrimental alterations. Despite advances in technology, off-target mutations compromise the safety and effectiveness of therapy(67).

Immune Response: Gene therapy faces challenges due to immune responses to delivery vectors, including viral and CRISPR components, which can lead to cell elimination and reduced treatment efficacy, especially in conditions like hemophilia **(68).**Furthermore, the immune system's reaction to viral capsids and the potential development of inhibitors pose additional challenges (69).

Precision And Safety: By employing lipid nanoparticles and biomaterials to target certain cells while lowering immunogenicity and off-target effects, advances in gene delivery methods are improving safety and precision (70).

Ethical And Regulatory Concerns: Balancing rapid CRISPR progress with safety/equity requires precision editing, ethical oversight, and flexible regulations to tackle technical and societal challenges (71).

DRUG THERAPIES IN GENETIC AND ACQUIRED DISEASES

Drugs like NSAIDs and anticonvulsants treat genetic disorders, though side effects and genetic variability limit their effectiveness (72). Biologics include gene-targeted therapies like CRISPR/Cas9, which address genetic mutations directly (73), Pharmacological treatments face limitations: incomplete genetic correction, adverse effects, and chronic dependency (74). Gene therapy presents a promising synergistic potential when combined with drug treatments. For instance, gene therapy can provide sustained therapeutic effects and potentially cure genetic disorders by introducing or correcting defective genes in patients' cells (75, 76) Such therapies are particularly effective in tackling monogenic diseases like inherited retinal disorders (77).

RATIONALE FOR COMBINATION THERAPY

Combining CRISPR with pharmacological agents presents several notable advantages, enhancing therapeutic interventions through various mechanisms such as overcoming resistance, improving efficacy, and enhancing delivery and expression efficiencies.

1. Overcoming Resistance And Improving Efficacy: By precisely altering genes implicated in resistance pathways, CRISPR technology can improve therapeutic efficacy and efficiently address drug resistance in chemotherapy treatments (78).

2. Enhancing Delivery And Gene Expression: Recent developments in gene editing applications demonstrate the effective use of adeno-associated viral vectors to deliver smaller CRISPR systems, allowing precise in vivo targeting of pharmaceutical drugs and improving therapeutic results (79). Additionally, combining CRISPR with liposome-based carriers enhances the delivery efficiency of CRISPR components, allowing for efficient editing of target genes (80).

3. Temporal Regulation And Dosage Control: When used with pharmaceuticals, CRISPR allows for precise temporal regulation of gene editing, optimizing therapeutic benefits through timing and control of editing (81). By targeting particular genetic components, improving drug administration, and advancing precision medicine

through biochemical benefits and sophisticated transport methods, CRISPR-pharmacological partnership offers promise for the treatment of disease (82).

How Drugs Enhance CRISPR Efficacy

Drugs like AZD7648 (DNA-PK inhibitor) and Polθ blockers boost CRISPR fidelity and integration by controlling DNA repair pathways (83),Co-delivering FAK siRNA and CRISPR components in nanoparticles has doubled CRISPR efficiency in tumor tissues, improving cellular uptake and penetration, thus enhancing editing efficiency in solid tumors (84). Advanced delivery methods (peptide/non-viral vectors) boost CRISPR precision and safety for therapeutic applications(85).The integration of drug-based approaches and innovative delivery strategies enhances CRISPR technology efficacy and precision, contributing to personalized medicine and targeted interventions for genetic disorders(86) as shown in Fig 3

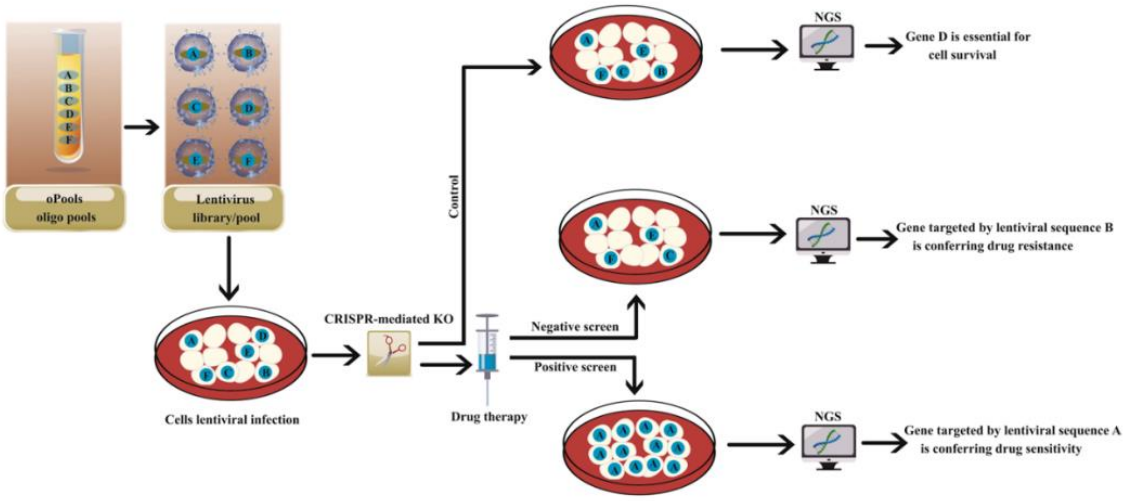


Fig 3: CRISPR screening uses pooled DNA oligos to target multiple genes, resulting in cell-infecting lentiviruses. Next-generation sequencing (NGS) can be used to identify genes that are present or absent, characterize resistance and sensitivity, and detect medication resistance or sensitivity(18)

SAFETY AND ETHICAL CONSIDERATIONS

1)Genotoxicity, Off-Target Effects, And Immune Reactions: CRISPR's curative potential demands improved precision, reduced risks, and ethical governance (87)

2)Regulatory Landscape for Gene-Drug Therapies: CRISPR regulations vary globally (strict in EU, flexible in US), requiring standardized safety/efficacy frameworks (88)

3)Ethical Concerns in Gene Editing: CRISPR's ease of use intensifies ethical debates over germline editing's societal and generational consequences (89)

Challenges And Limitations

1)Technical, Biological, and Cost Challenges: CRISPR delivery via nanomedicine shows promise yet requires optimization for safety, efficacy, and affordability to ensure equitable access (90)

2)Scalability and Manufacturing: CRISPR's clinical scalability faces hurdles in manufacturing, IP disputes, and regulatory uncertainties, despite its transformative potential in biotechnology (91)

Future Directions and Emerging Trends

The next generation of CRISPR systems including Cas12 and Cas13 demonstrate superior precision and expanded editing capabilities (e.g., RNA targeting), reducing off-target effects and

broadening therapeutic potential for complex diseases (92) Concurrently, AI-driven therapy design is revolutionizing precision medicine by optimizing gene-editing strategies through predictive modeling, enabling patient-specific treatments(93) A groundbreaking frontier is CRISPR's application in polygenic diseases (e.g., diabetes, cardiovascular disorders), where multiplexed editing could simultaneously modulate disease-associated gene networks, though this demands advanced delivery systems and rigorous regulatory evaluation(94)

CONCLUSION

CRISPR-drug combinations enhance gene therapy precision and efficacy for genetic disorders and cancer, but require improved delivery, reduced off-target effects, and ethical solutions for clinical translation

REFERENCES

1. Maldonado R, Jalil S, Wartiovaara K. Curative gene therapies for rare diseases. Journal of Community Genetics. 2021;12(2):267-76.
2. Lykken EA, Shyng C, Edwards RJ, Rozenberg A, Gray SJ. Recent progress and considerations for AAV gene therapies targeting the central nervous system. Journal of neurodevelopmental disorders. 2018;10:1-10.
3. Jayarajan V, Kounatidou E, Qasim W, Di WL. Ex vivo gene modification therapy for genetic skin diseases—Recent advances in gene modification technologies and delivery. Experimental Dermatology. 2021;30(7):887-96.
4. Fuller-Carter PI, Basiri H, Harvey AR, Carvalho LS. Focused update on AAV-based gene therapy clinical trials for inherited retinal degeneration. BioDrugs. 2020;34:763-81.
5. Godbout K, Tremblay JP. Prime editing for human gene therapy: where are we now? Cells. 2023;12(4):536.
6. Shuvalov O, Petukhov A, Daks A, Fedorova O, Ermakov A, Melino G, et al. Current genome editing tools in gene therapy: new approaches to treat cancer. Current gene therapy. 2015;15(5):511-29.
7. Roma-Rodrigues C, Rivas-García L, Baptista PV, Fernandes AR. Gene therapy in cancer treatment: why go nano? Pharmaceutics. 2020;12(3):233.
8. Bottai G, Truffi M, Corsi F, Santarpia L. Progress in nonviral gene therapy for breast cancer and what comes next? Expert Opinion on Biological Therapy. 2017;17(5):595-611.
9. Touchefeu Y, Harrington K, Galmiche JP, Vassaux G. Gene therapy, recent developments and future prospects in gastrointestinal oncology. Alimentary pharmacology & therapeutics. 2010;32(8):953-68.
10. Youssef E, Fletcher B, Palmer D. Enhancing precision in cancer treatment: the role of gene therapy and immune modulation in oncology. Frontiers in Medicine. 2025;11:1527600.
11. Dranoff G. Cancer gene therapy: connecting basic research with clinical inquiry. Journal of clinical oncology. 1998;16(7):2548-56.
12. Lu X, Zhang M, Li G, Zhang S, Zhang J, Fu X, et al. Applications and research advances in the delivery of CRISPR/Cas9 systems for the treatment of inherited diseases. International journal of molecular sciences. 2023;24(17):13202.
13. Xu W, Zhang S, Qin H, Yao K. From bench to bedside: cutting-edge applications of base editing and prime editing in precision medicine. Journal of Translational Medicine. 2024;22(1):1133.
14. Macarrón Palacios A, Korus P, Wilkens BG, Heshmatpour N, Patnaik SR. Revolutionizing in vivo therapy with CRISPR/Cas genome editing: breakthroughs, opportunities and challenges. Frontiers in Genome Editing. 2024;6:1342193.

15. Mollanoori H, Teimourian S. Therapeutic applications of CRISPR/Cas9 system in gene therapy. Biotechnology letters. 2018;40:907-14.
16. Ding S, Liu J, Han X, Tang M. CRISPR/Cas9-mediated genome editing in cancer therapy. International journal of molecular sciences. 2023;24(22):16325.
17. Sankar A, YS RK, Singh A, Roy R, Shukla R, Verma B. Next-generation therapeutics for rare genetic disorders. Mutagenesis. 2024;39(3):157-71.
18. Shojaei Baghini S, Gardanova ZR, Abadi SAH, Zaman BA, İlhan A, Shomali N, et al. CRISPR/Cas9 application in cancer therapy: a pioneering genome editing tool. Cellular & Molecular Biology Letters. 2022;27(1):35.
19. Van Hasselt JC, Iyengar R. Systems pharmacology: defining the interactions of drug combinations. Annual review of pharmacology and toxicology. 2019;59(1):21-40.
20. Halpern B, Mancini MC. Safety assessment of combination therapies in the treatment of obesity: focus on naltrexone/bupropion extended release and phentermine-topiramate extended release. Expert Opinion on Drug Safety. 2017;16(1):27-39.
21. Koch G, Walz A, Lahu G, Schropp J. Modeling of tumor growth and anticancer effects of combination therapy. Journal of pharmacokinetics and pharmacodynamics. 2009;36:179-97.
22. Siddiqui-Jain A, Bliesath J, Macalino D, Omori M, Huser N, Streiner N, et al. CK2 inhibitor CX-4945 suppresses DNA repair response triggered by DNA-targeted anticancer drugs and augments efficacy: mechanistic rationale for drug combination therapy. Molecular cancer therapeutics. 2012;11(4):994-1005.
23. Coletti R, Leonardelli L, Parolo S, Marchetti L. A QSP model of prostate cancer immunotherapy to identify effective combination therapies. Scientific reports. 2020;10(1):9063.
24. Ashby LS, Ryken TC. Management of malignant glioma: steady progress with multimodal approaches. Neurosurgical focus. 2006;20(4):E3.
25. Zakrzewska M, Burmistrz M. Mechanisms regulating the CRISPR-Cas systems. Frontiers in microbiology. 2023;14:1060337.
26. Wang JY, Pausch P, Doudna JA. Structural biology of CRISPR–Cas immunity and genome editing enzymes. Nature Reviews Microbiology. 2022;20(11):641-56.
27. Anton T, Karg E, Bultmann S. Applications of the CRISPR/Cas system beyond gene editing. Biology Methods and Protocols. 2018;3(1):bpy002.
28. Hwarari D, Radani Y, Ke Y, Chen J, Yang L. CRISPR/Cas genome editing in plants: mechanisms, applications, and overcoming bottlenecks. Functional & Integrative Genomics. 2024;24(2):50.
29. Liu Q, Zhang H, Huang X. Anti-CRISPR proteins targeting the CRISPR-Cas system enrich the toolkit for genetic engineering. The FEBS journal. 2020;287(4):626-44.
30. Kumita W, Sato K, Suzuki Y, Kurotaki Y, Harada T, Zhou Y, et al. Efficient generation of Knock-in/Knock-out marmoset embryo via CRISPR/Cas9 gene editing. Scientific reports. 2019;9(1):12719.
31. Kim J, Lee S, Baek K, Jin E. Site-specific gene knock-out and on-site heterologous gene overexpression in Chlamydomonas reinhardtii via a CRISPR-Cas9-mediated knock-in method. Frontiers in plant science. 2020;11:306.
32. Kantor A, McClements ME, MacLaren RE. CRISPR-Cas9 DNA base-editing and prime-editing. International journal of molecular sciences. 2020;21(17):6240.
33. Yu S-Y, Birkenshaw A, Thomson T, Carlaw T, Zhang L-H, Ross CJ. Increasing the targeting scope of CRISPR base editing system beyond NGG. The Crispr Journal. 2022;5(2):187-202.

34. Hillary VE, Ceasar SA. Prime editing in plants and mammalian cells: mechanism, achievements, limitations, and future prospects. BioEssays. 2022;44(9):2200032.
35. Wei J, Li Y. CRISPR-based gene editing technology and its application in microbial engineering. Engineering Microbiology. 2023;3(4):100101.
36. Prado GS, Rocha DC, Santos LNd, Contiliani DF, Nobile PM, Martinati-Schenk JC, et al. CRISPR technology towards genome editing of the perennial and semi-perennial crops citrus, coffee and sugarcane. Frontiers in Plant Science. 2024;14:1331258.
37. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. Science. 2013;339(6121):819-23.
38. Jubair L, Lam AK, Fallaha S, McMillan NAJ. CRISPR/Cas9-loaded stealth liposomes effectively cleared established HPV16-driven tumours in syngeneic mice. PLoS One. 2021;16(1):e0223288.
39. Ebina H, Misawa N, Kanemura Y, Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci Rep. 2013;3:2510.
40. Li X, Guo M, Hou B, Zheng B, Wang Z, Huang M, et al. CRISPR/Cas9 nanoeditor of double knockout large fragments of E6 and E7 oncogenes for reversing drugs resistance in cervical cancer. J Nanobiotechnology. 2021;19(1):231.
41. Lin SR, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC, et al. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. Mol Ther Nucleic Acids. 2014;3(8):e186.
42. Liao HK, Gu Y, Diaz A, Marlett J, Takahashi Y, Li M, et al. Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nat Commun. 2015;6:6413.
43. Hou P, Chen S, Wang S, Yu X, Chen Y, Jiang M, et al. Genome editing of CXCR4 by CRISPR/cas9 confers cells resistant to HIV-1 infection. Sci Rep. 2015;5:15577.
44. Zhen S, Lu J, Liu YH, Chen W, Li X. Synergistic antitumor effect on cervical cancer by rational combination of PD1 blockade and CRISPR-Cas9-mediated HPV knockout. Cancer Gene Ther. 2020;27(3-4):168-78.
45. Lao YH, Li M, Gao MA, Shao D, Chi CW, Huang D, et al. HPV Oncogene Manipulation Using Nonvirally Delivered CRISPR/Cas9 or Natronobacterium gregoryi Argonaute. Adv Sci (Weinh). 2018;5(7):1700540.
46. Nguyen H, Wilson H, Jayakumar S, Kulkarni V, Kulkarni S. Efficient Inhibition of HIV Using CRISPR/Cas13d Nuclease System. Viruses. 2021;13(9).
47. Hong M, Clubb JD, Chen YY. Engineering CAR-T Cells for Next-Generation Cancer Therapy. Cancer Cell. 2020;38(4):473-88.
48. Glienke W, Esser R, Priesner C, Suerth JD, Schambach A, Wels WS, et al. Advantages and applications of CAR-expressing natural killer cells. Front Pharmacol. 2015;6:21.
49. Haji-Fatahaliha M, Hosseini M, Akbarian A, Sadreddini S, Jadidi-Niaragh F, Yousefi M. CAR-modified T-cell therapy for cancer: an updated review. Artif Cells Nanomed Biotechnol. 2016;44(6):1339-49.
50. Han D, Xu Z, Zhuang Y, Ye Z, Qian Q. Current Progress in CAR-T Cell Therapy for Hematological Malignancies. J Cancer. 2021;12(2):326-34.
51. Qu J, Mei Q, Chen L, Zhou J. Chimeric antigen receptor (CAR)-T-cell therapy in non-small-cell lung cancer (NSCLC): current status and future perspectives. Cancer Immunol Immunother. 2021;70(3):619-31.
52. Corti C, Venetis K, Sajjadi E, Zattoni L, Curigliano G, Fusco N. CAR-T cell therapy for triple-negative breast cancer and other solid tumors: preclinical and clinical progress. Expert Opin Investig Drugs. 2022;31(6):593-605.
53. Zhao J, Lin Q, Song Y, Liu D. Universal CARs, universal T cells, and universal CAR T cells. J Hematol Oncol. 2018;11(1):132.

54. Budi HS, Ahmad FN, Achmad H, Ansari MJ, Mikhailova MV, Suksatan W, et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor (CAR) for tumor immunotherapy; recent progress. Stem Cell Res Ther. 2022;13(1):40.
55. Marofi F, Tahmasebi S, Rahman HS, Kaigorodov D, Markov A, Yumashev AV, et al. Any closer to successful therapy of multiple myeloma? CAR-T cell is a good reason for optimism. Stem Cell Res Ther. 2021;12:1-21.
56. Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest. 2016;126(8):3130-44.
57. Marofi F, Rahman HS, Thangavelu L, Dorofeev A, Bayas-Morejón F, Shirafkan N, et al. Renaissance of armored immune effector cells, CAR-NK cells, brings the higher hope for successful cancer therapy. Stem Cell Res Ther. 2021;12(1):200.
58. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018;359(6382):1361-5.
59. Asmamaw Mengstie M. Viral vectors for the in vivo delivery of CRISPR components: advances and challenges. Frontiers in bioengineering and biotechnology. 2022;10:895713.
60. Xu X, Wan T, Xin H, Li D, Pan H, Wu J, et al. Delivery of CRISPR/Cas9 for therapeutic genome editing. The journal of gene medicine. 2019;21(7):e3107.
61. Ren S, Wang M, Wang C, Wang Y, Sun C, Zeng Z, et al. Application of non-viral vectors in drug delivery and gene therapy. Polymers. 2021;13(19):3307.
62. Rakoczy K, Kisielewska M, Sędzik M, Jonderko L, Celińska J, Sauer N, et al. Electroporation in clinical applications—the potential of gene electrotransfer and electrochemotherapy. Applied Sciences. 2022;12(21):10821.
63. Jacob S, Nair AB, Shah J, Gupta S, Boddu SH, Sreeharsha N, et al. Lipid nanoparticles as a promising drug delivery carrier for topical ocular therapy—an overview on recent advances. Pharmaceutics. 2022;14(3):533.
64. Fukuta T, Kogure K. Biomimetic nanoparticle drug delivery systems to overcome biological barriers for therapeutic applications. Chemical and Pharmaceutical Bulletin. 2022;70(5):334-40.
65. Rittiner JE, Moncalvo M, Chiba-Falek O, Kantor B. Gene-editing technologies paired with viral vectors for translational research into neurodegenerative diseases. Frontiers in molecular neuroscience. 2020;13:148.
66. Sung YK, Kim S. Recent advances in the development of gene delivery systems. Biomaterials research. 2019;23(1):8.
67. Chehelgerdi M, Chehelgerdi M, Khorramian-Ghahfarokhi M, Shafieizadeh M, Mahmoudi E, Eskandari F, et al. Comprehensive review of CRISPR-based gene editing: mechanisms, challenges, and applications in cancer therapy. Molecular cancer. 2024;23(1):9.
68. Arruda VR, Samelson-Jones BJ. Obstacles and future of gene therapy for hemophilia. Expert opinion on orphan drugs. 2015;3(9):997-1010.
69. Lei T, Wang Y, Zhang Y, Yang Y, Cao J, Huang J, et al. Leveraging CRISPR gene editing technology to optimize the efficacy, safety and accessibility of CAR T-cell therapy. Leukemia. 2024:1-27.
70. Iqbal Z, Rehman K, Xia J, Shabbir M, Zaman M, Liang Y, et al. Biomaterial-assisted targeted and controlled delivery of CRISPR/Cas9 for precise gene editing. Biomaterials science. 2023;11(11):3762-83.
71. Bailey SR, Maus MV. Gene editing for immune cell therapies. Nature biotechnology. 2019;37(12):1425-34.

72. Belyaeva II, Subbotina AG, Eremenko II, Tarasov VV, Chubarev VN, Schiöth HB, et al. Pharmacogenetics in primary headache disorders. Frontiers in Pharmacology. 2022;12:820214.
73. Hussen B, Ismael B, Abdulla S, Jamal N, Mustafa S, Najmalddin Z, et al. Molecular mechanisms of breast Cancer drug resistance and CRISPR/Cas9 strategies to overcome. BioMed Target J. 2024;2(2):1-20.
74. Blau HM, Springer ML. Gene therapy—a novel form of drug delivery. New England Journal of Medicine. 1995;333(18):1204-7.
75. Berger J, Pujol A, Aubourg P, Forss-Petter S. Current and future pharmacological treatment strategies in X-linked adrenoleukodystrophy. Brain pathology. 2010;20(4):845-56.
76. Bannwarth B. Drug-induced musculoskeletal disorders. Drug Saf. 2007;30(1):27-46.
77. Nuzbrokh Y, Ragi SD, Tsang SH. Gene therapy for inherited retinal diseases. Annals of Translational Medicine. 2021;9(15):1278.
78. Wang S-W, Gao C, Zheng Y-M, Yi L, Lu J-C, Huang X-Y, et al. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Molecular cancer. 2022;21(1):57.
79. Cui T, Cai B, Tian Y, Liu X, Liang C, Gao Q, et al. Therapeutic In Vivo Gene Editing Achieved by a Hypercompact CRISPR-Cas12f1 System Delivered with All-in-One Adeno-Associated Virus. Advanced Science. 2024;11(19):2308095.
80. Yin X, Harmancey R, McPherson DD, Kim H, Huang S-L. Liposome-based carriers for CRISPR genome editing. International Journal of Molecular Sciences. 2023;24(16):12844.
81. Brackett NF, Pomés A, Chapman MD. New Frontiers: Precise Editing of Allergen Genes Using CRISPR. Front Allergy. 2021;2:821107.
82. Zhang S, Wang Y, Mao D, Wang Y, Zhang H, Pan Y, et al. Current trends of clinical trials involving CRISPR/Cas systems. Front Med (Lausanne). 2023;10:1292452.
83. Wimberger S, Akrap N, Firth M, Brengdahl J, Engberg S, Schwinn MK, et al. Simultaneous inhibition of DNA-PK and Polθ improves integration efficiency and precision of genome editing. Nature communications. 2023;14(1):4761.
84. Zhang D, Wang G, Yu X, Wei T, Farbiak L, Johnson LT, et al. Enhancing CRISPR/Cas gene editing through modulating cellular mechanical properties for cancer therapy. Nature nanotechnology. 2022;17(7):777-87.
85. Shalaby K, Aouida M, El-Agnaf O. Tissue-specific delivery of CRISPR therapeutics: Strategies and mechanisms of non-viral vectors. International Journal of Molecular Sciences. 2020;21(19):7353.
86. Alagoz M, Kherad N. Advance genome editing technologies in the treatment of human diseases: CRISPR therapy. International journal of molecular medicine. 2020;46(2):521-34.
87. Han HA, Pang JKS, Soh B-S. Mitigating off-target effects in CRISPR/Cas9-mediated in vivo gene editing. Journal of Molecular Medicine. 2020;98(5):615-32.
88. Irfan M, Majeed H, Iftikhar T, Ravi PK. A review on molecular scissoring with CRISPR/Cas9 genome editing technology. Toxicology Research. 2024;13(4):tfae105.
89. Rodriguez E. Ethical issues in genome editing using Crispr/Cas9 system. Journal of Clinical Research and Bioethics. 2016;7(2).
90. Sioson VA, Kim M, Joo J. Challenges in delivery systems for CRISPR-based genome editing and opportunities of nanomedicine. Biomedical Engineering Letters. 2021;11:217-33.
91. Schacker M, Seimetz D. From fiction to science: clinical potentials and regulatory considerations of gene editing. Clinical and translational medicine. 2019;8(1):27.
92. Pacesa M, Pelea O, Jinek M. Past, present, and future of CRISPR genome editing technologies. Cell. 2024;187(5):1076-100.

93. Kolanu ND. CRISPR–Cas9 gene editing: curing genetic diseases by inherited epigenetic modifications. Global Medical Genetics. 2024;11(01):113-22.
94. Garg P, Singhal G, Pareek S, Kulkarni P, Horne D, Nath A, et al. Unveiling the potential of gene editing techniques in revolutionizing cancer treatment: A comprehensive overview. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2024:189233.