

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

Mehwish Nisar^{*1}, Zufi Shad², Sidra Siddiqui³, Iqra Haider⁴, Tahmina Maqbool⁵

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

¹sidra.siddiqui@hamdard.edu.pk

^{*1}<https://orcid.org/0009-0000-5587-781X>

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Corresponding Author: *

Mehwish Nisar

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

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

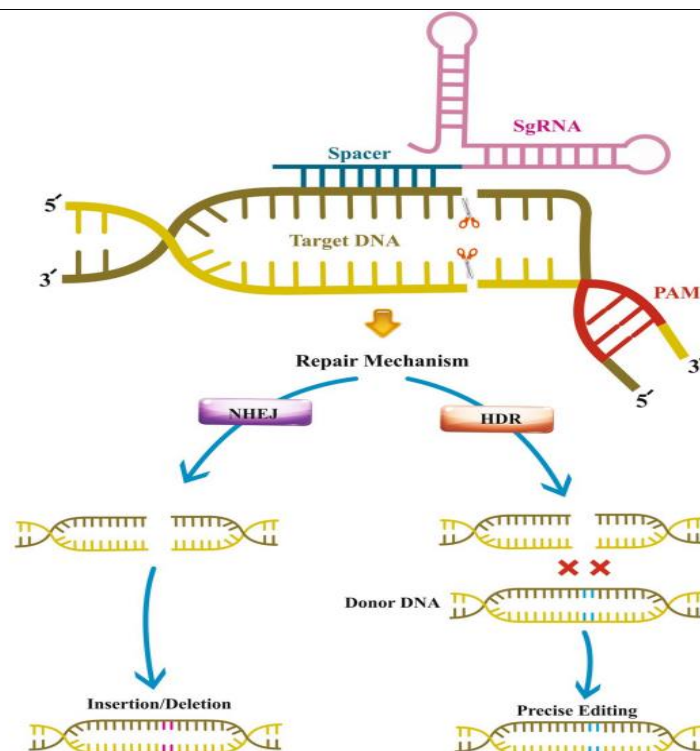


Fig. 1. The mechanisms of action of the CRISPR/Cas9 system, including nonhomologous end-joining (NHEJ), homology-directed repair (HDR), single-guide RNA (sgRNA), and protospacer adjacent motif (PAM),(18)

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	Efficient 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 effect 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.

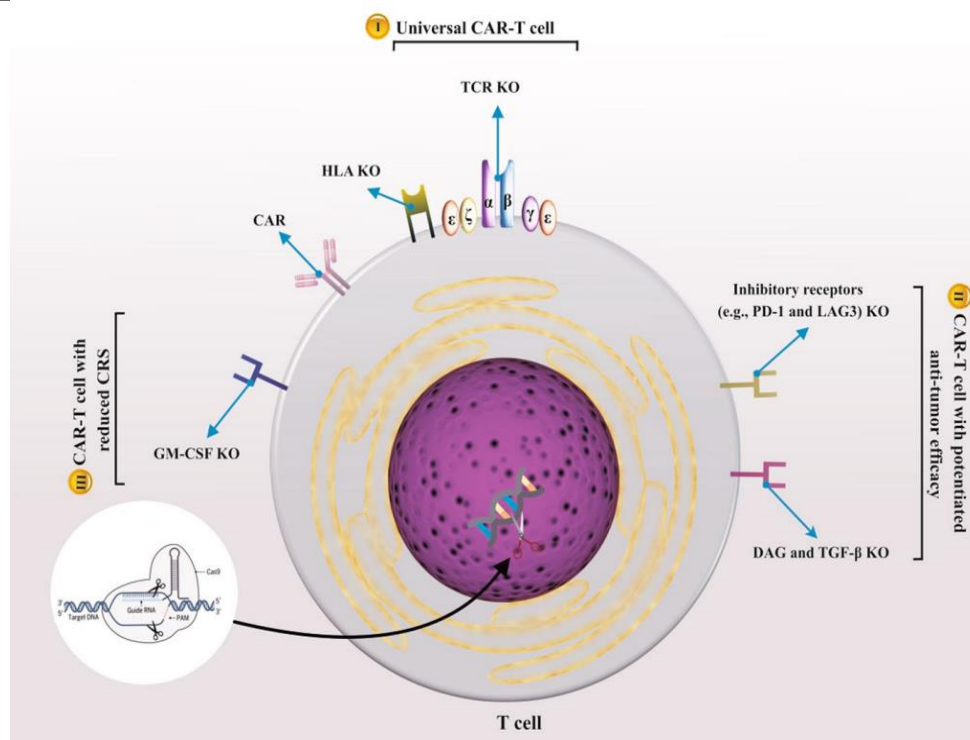


Fig 2: Utilizing a range of genes, including KO, HLA, GM-CSF, TCR, LAG-3, TGF-βR, DAG, and PD1, CRISPR/Cas9 enables future CAR-T cell production.(18)

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

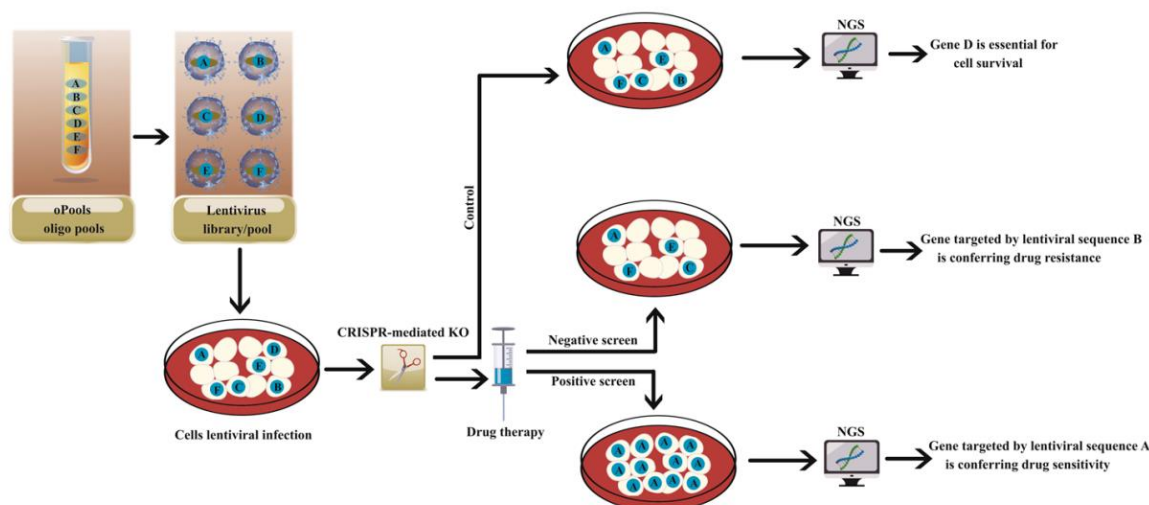


Fig 3: CRISPR screening uses pooled DNA oligos to target multiple genes, resulting in cell-infecting lentiviruses. Next-generation sequencing (NGS) can be used to identify genes that are present or absent, characterize resistance and sensitivity, and detect medication resistance or sensitivity(18)

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.

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