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Advances in Clinical Research on CRISPR-Cas9 for the Treatment of Common Monogenic Diseases

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Abstract:

CRISPR-Cas9 gene editing technology has achieved milestone breakthroughs in the treatment of monogenic hereditary diseases. We systematically review the latest clinical research progress of this technology in treating common monogenic disorders, summarizing key efficacy data, safety outcomes, and current major obstacles. On this basis, we analyze the shift in application logic when extending from monogenic diseases to specific cancers and long COVID syndrome, that is, moving from defect repair to engineering modification and immunomodulation. For cancer, we discuss the prospects of CRISPR in CAR-T enhancement and oncogene disruption, as well as challenges such as tumor heterogeneity and delivery bottlenecks. For long COVID, we explore its value as a research tool and the prospects for direct therapy. Finally, we propose strategies to address common challenges including delivery optimization, off-target detection standardization, and ethical regulation. The success of CRISPR-Cas9 in monogenic diseases represents the prelude to precision medicine, whereas conquering complex diseases will require systematic leaps in target discovery, delivery technology, and safety profiles.

Keywords: CRISPR-Cas9; monogenic diseases; gene therapy; precision medicine; cancer immunotherapy; long COVID

1. Introduction

The core objective of precision medicine is to develop personalized prevention, diagnosis, and treatment strategies based on an individual's genetic background, environmental exposures, and lifestyle characteristics, thereby facilitating a paradigm shift from population statistics-based evidence-based medicine to molecular-level individualized intervention. In the field of hereditary diseases, thousands of monogenic disorders affect millions of people worldwide, yet the vast majority lack etiological therapies. Conventional treatments such as small-molecule drugs, protein replacement, and organ transplantation only alleviate

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symptoms without correcting the underlying genetic defects. Taking sickle cell disease and hereditary amyloidosis as examples, long-term blood transfusion, hydroxyurea, or liver transplantation may partially relieve clinical manifestations but cannot eliminate the continuous production of aberrant gene products, and are accompanied by significant risks and lifelong management burdens. Therefore, the development of technologies capable of directly repairing or compensating for pathogenic mutations has become a central challenge of the precision medicine era.

The advent of CRISPR-Cas9 technology has provided a revolutionary tool to address this challenge. Clustered regularly interspaced short palindromic repeats (CRISPR) originate from the adaptive immune system of bacteria and archaea, functioning to recognize and cleave foreign DNA [1]. By designing a synthetic single guide RNA (sgRNA) complementary to the target DNA, the Cas9 protein can be precisely directed to any genomic locus to induce site-specific double-strand breaks. Compared with earlier gene-editing tools such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 requires only a change of the approximately 20-nucleotide guide sequence within the sgRNA to redirect editing to a new target. The design cycle is shortened to days or even hours, the cost is reduced to a few hundred dollars, and the system exhibits high efficiency and specificity in diverse cell types [2]. These characteristics have established CRISPR-Cas9 as a core platform for gene therapy and also led to Charpentier and Doudna receiving the Nobel Prize in Chemistry in 2020. Among its many applications, monogenic diseases represent the most direct and promising entry point for CRISPR-Cas9. Monogenic disorders are caused by one or a few mutations at a single genetic locus, with clear causal relationships [3]. Typical examples include sickle cell disease (SCD), β-thalassemia (TDT), and hereditary transthyretin amyloidosis (ATTR). The pathogenic logic is highly linear—"one gene defect leads to one disease"—implying that repairing that gene could, in theory, achieve a one-time cure. In contrast, complex diseases such as cancer and long COVID involve multiple genetic, environmental, and epigenetic factors, making it difficult to achieve ideal therapeutic effects by targeting a single gene. The advantages of monogenic diseases—single target, clear causality—make them ideal models for validating the clinical feasibility of CRISPR.

As clinical trials for monogenic diseases progress, a forward-looking question naturally emerges: can the successful experience with CRISPR be extended to more complex diseases? Cancer, as a polygenic and highly heterogeneous disease, accumulates numerous driver mutations in its genome accompanied by chromosomal structural variations; long COVID, as a recently recognized syndrome, involves multiple hypotheses such as persistent viral presence and immune dysregulation, lacking a clear and unified genetic target. How should editing strategies be designed when targets are unclear or non-singular? Can existing delivery systems meet the demands of precise editing in complex tissues? How does heterogeneity affect therapeutic outcomes? Based on these questions, this review aims to systematically summarize the latest clinical progress of CRISPR-Cas9 in treating common monogenic diseases, analyze the theoretical and practical challenges when extending this technology to specific cancers and long COVID, and explore potential solutions from technical, safety, and ethical perspectives.

2. Clinical Research Advances of CRISPR-Cas9 in the Treatment of Common Monogenic Diseases

2.1 Monogenic Disorders of the Hematopoietic System

Hemoglobinopathies are among the most common and burdensome monogenic diseases globally, with SCD and TDT as representative examples. SCD is caused by a point mutation at codon 6 of the HBB gene, leading to polymerization of abnormal hemoglobin (HbS) and resulting in erythrocyte sickling and microvascular occlusion [4]. TDT is caused by deletions or nonsense mutations in the HBB gene, leading to insufficient synthesis of β-globin chains and severe anemia. Conventional therapies only alleviate symptoms, while allogeneic hematopoietic stem cell transplantation is limited by donor availability and the risk of graft-versus-host disease. Therefore, a gene-editing strategy based on autologous hematopoietic stem cells (HSCs) has emerged as a curative approach. Instead of directly repairing the mutated HBB gene, this strategy uses CRISPR-Cas9 to disrupt the erythroid-specific enhancer of the BCL11A gene, thereby relieving transcriptional repression of the fetal hemoglobin (HbF)-encoding genes HBG1/2 and re-inducing HbF synthesis to compensate for defective adult hemoglobin [5]. This approach employs the non-homologous end joining pathway, achieving high editing efficiency without being restricted to a specific mutation type.

Key clinical trials (CLIMB-111, CLIMB-121) have shown that among more than 75 patients with TDT, over 90% achieved transfusion independence after a median

follow-up of 24 months; in over 40 patients with SCD, the annual incidence of vaso-occlusive crises (VOCs) decreased from 3.5 to 0 after treatment [6]. HbF accounted for more than 40% of total hemoglobin, and the efficacy lasted beyond 36 months. Regarding safety, no off-target editing events were detected, and adverse events were mainly known toxicities from the myeloablative conditioning regimen. Current major challenges include high cost and poor accessibility; the development of allogeneic universal products and reduction of manufacturing costs are urgently needed.

Table 1. Summary of representative clinical trials of CRISPR-Cas9 for monogenic diseases

Disease	Target Gene/Regulatory Element	Editing Strategy	Delivery System	Key Efficacy Outcomes	Major Safety Outcomes	Clinical Trial Phase
SCD	BCL11A enhancer	Knockout	Ex vivo / Electroporation	Transfusion independence, no VOC	Off-target negative, myeloablative toxicity	Phase I/II
TDT	BCL11A enhancer	Knockout	Ex vivo / Electroporation	Transfusion independence >90%	Same as SCD	Phase I/II
ATTR Amyloidosis	TTR gene	Knockout	In vivo / LNP	Serum TTR reduction >80%	Mild liver enzyme elevation	Phase I
LCA10	CEP290 mutation	Knockout/Repair	In vivo / AAV5	Partial visual function improvement	Immune response	Phase I/II

2.2 Exploration of Other Monogenic Diseases

ATTR is caused by mutations in the TTR gene, leading to pathological deposition of abnormal proteins in tissues [7]. The phase I NTLA-2001 trial in 2021 provided the first demonstration that intravenous administration of lipid nanoparticle-encapsulated CRISPR-Cas9 can be taken up by hepatocytes and achieve TTR gene knockout. In the high-dose group (1.0 mg/kg), serum TTR levels were reduced by 80%-90%, with efficacy sustained for more than 12 months, without the need for ex vivo manipulation or myeloablative conditioning. However, LNP delivery is currently limited to the liver, and efficient extrahepatic targeting strategies remain lacking.

LCA10 is caused by mutations in the CEP290 gene, whose coding region spans 7.5 kb, exceeding the packaging capacity of AAV. The EDIT-101 trial used AAV5 to deliver SaCas9 and a sgRNA into the subretinal space. Among the 14 treated patients, some showed improvements in light sensitivity and visual field, yet significant inter-individual variability in efficacy was observed [8]. Challenges include immune responses induced by persistent AAV-mediated Cas9 expression, limited retinal coverage achieved by subretinal injection, and insufficient editing efficiency to restore normal function [9]. Furthermore, CRISPR-based research for monogenic diseases such as DMD and Huntington's disease remains at the preclinical stage, with in vivo delivery to skeletal muscles throughout the body or to the central nervous system representing a major obstacle.

2.3 Research Review

Several generalizable insights can be drawn from the above clinical progress. First, a well-defined pathogenic gene and a feasible editing strategy are prerequisites for success. The disruption of the BCL11A enhancer in SCD/TDT and the knockout of the TTR gene in ATTR both build upon a clear understanding of disease causality, and employ the relatively mature and efficient NHEJ pathway, thereby circumventing the low efficiency of homology-directed repair. Second, a robust delivery system is critical for clinical translation. The two currently clinically validated delivery paradigms—ex vivo editing of autologous HSCs and in vivo LNP delivery to the liver—represent the forefront of the field. Ex vivo editing enables quality control of the edited cells but requires myeloablative conditioning; in vivo editing is simpler and avoids myeloablative toxicity, but its targeting range is limited. Third, safety and off-target effects remain unresolved core issues. Although current detection methods have not identified significant off-target events, long-term follow-up is still necessary to assess potential carcinogenic risks, immunogenicity, and durability of editing. Fourth, cost and accessibility severely constrain the broad applicability of the technology. The highly personalized nature, precision manufacturing requirements, and need for advanced medical facilities associated with current therapies render them inaccessible to the overwhelming majority of patients in low- and middle-income countries.

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3. From Monogenic Diseases to Complex Diseases: Shifts in Application Logic and Scientific Foundations

3.1 Essential Differences Between Monogenic and Complex Diseases

Monogenic diseases and complex diseases exhibit fundamental differences in genetic architecture, pathological mechanisms, and intervention strategies, which determine the non-linear increase in difficulty when extending CRISPR-Cas9 technology from the former to the latter. Monogenic diseases follow Mendelian inheritance patterns: a specific mutation in a single gene has high penetrance, and the causal chain is clear and predictable [10]. In contrast, complex diseases typically involve the accumulation of polygenic minor-effect mutations, dynamic changes in epigenetic modifications, and interactions with environmental factors, presenting high genetic heterogeneity and phenotypic plasticity. Taking solid tumors as an example, a single tumor can harbor dozens to hundreds of gene mutations, and the mutational profiles of different patients rarely overlap. Long COVID lacks a clear pathogenic gene; its onset and progression are more closely associated with persistent viral presence, dysregulation of immune networks, and individual susceptibility, with no unified molecular target. In monogenic diseases, the CRISPR editing target is clear and singular—knocking out or repairing a single site can achieve significant therapeutic efficacy. In complex diseases, however, one must confront a series of fundamental questions: "which gene or genes should be edited?", "how to cope with subclonal diversity?", and "how to adapt to disease evolution?". Table 2 compares the key features of the two disease categories across multiple dimensions.

Table 2. Comparison of key features between monogenic diseases and complex diseases

Dimension	Monogenic Diseases	Complex Diseases
Genetic pattern	Single gene mutation, high penetrance	Combined effects of polygenic/epigenetic/environmental factors
Number of causative factors	One (or a few)	Dozens to hundreds, dynamically changing
Target clarity	Highly clear, direct causality	Highly heterogeneous, lacking universal targets
Interpatient heterogeneity	Low	Extremely high
Intralesional heterogeneity	None	Significant (tumor subclones)
Main editing strategy	Repair or knock out the defective gene	Engineer immune cells / disrupt oncogenes / modulate immune networks
Major bottlenecks for success	Delivery efficiency, offtarget safety	Target discovery + delivery + tumor microenvironment + immune complexity
Representative diseases	SCD, TDT, ATTR, LCA10	Solid tumors, hematologic malignancies, long COVID

3.2 The Shift in Application Logic: From Repair to Engineering

In monogenic diseases, the core strategy is "error correction"—directly repairing the pathogenic mutation or modulating its expression level to restore physiological function. This strategy is built on two premises: first, the loss-of-function or gain-of-toxicity of the defective gene has been thoroughly validated; second, the edited cells or tissues can maintain normal physiological function over the long term. In cancer, by contrast, the vast majority of mutations carried by tumor cells are "passenger mutations", and only a few "driver mutations" play key roles in tumor initiation and progression. Moreover, driver mutations often involve activation of proto-oncogenes or inactivation of tumor suppressor genes. Therefore, the logic of CRISPR application in cancer is one of disruption or enhancement. For example, in CAR-T therapy, CRISPR is used to knock out the endogenous PD-1 gene in T cells to relieve immune suppression, or to knock in a chimeric antigen receptor to confer new recognition capabilities upon the T cells. In direct anti-tumor strategies, CRISPR is used to disrupt oncogenic fusion genes or mutant sites in tumor suppressor genes. For long COVID, although the pathological mechanisms have not been fully elucidated, hypotheses involve persistent viral reservoirs and immune dysregulation. Accordingly, potential strategies may include using CRISPR-Cas9 or Cas13 to destroy integrated viral genomes, or editing genes that regulate T-cell function to restore immune homeostasis.

4. Prospects and Challenges of CRIS-

PR-Cas9 in Specific Cancers and Long COVID

4.1 Specific Cancers

4.1.1 Application Prospects

Cancer represents the primary domain for extending CRISPR-Cas9 technology beyond monogenic diseases. The most promising directions for clinical translation include engineering immune cells (CAR-T therapy), direct disruption of oncogenes, and the use of CRISPR libraries to screen for novel drug targets.

(1) Engineering immune cells (enhanced CAR-T)

CAR-T therapy has been successfully used to treat B-cell malignancies, but it faces two major bottlenecks: first, T cells are prone to exhaustion and inhibition by the tumor microenvironment (especially the PD-1/PD-L1 pathway); second, the production of autologous CAR-T cells is time-consuming and costly. CRISPR-Cas9 can address both issues simultaneously. For example, knockout of the PD-1 gene in T cells blocks immune checkpoint inhibition and enhances the anti-tumor activity of CAR-T cells; knockout of the T-cell receptor (TCR) and HLA-I molecules enables the generation of universal CAR-T (UCAR-T) cells that can be used "off-the-shelf", greatly reducing time and cost. Moreover, knock-in of a safety switch (e.g., inducible caspase 9, iCasp9) can induce T-cell apoptosis upon severe cytokine release syndrome, improving treatment safety. Several phase I clinical trials (e.g., at the University of Pennsylvania, Stanford University, etc.) have preliminarily demonstrated the feasibility and tolerability of CRISPR-edited CAR-T cells in refractory B-cell lymphoma and multiple myeloma, with some patients achieving complete remission.

(2) Direct disruption of oncogenes

For tumors driven by a single oncogenic fusion gene, CRISPR can be directly targeted to disrupt the fusion site. The most typical example is the BCR-ABL fusion gene—formed by translocation between chromosomes 9 and 22—which causes chronic myeloid leukemia (CML). By designing a specific sgRNA that spans the BCR-ABL fusion breakpoint, double-strand breaks can be induced exclusively at the fusion gene without affecting the normal alleles. Preclinical studies have confirmed that this strategy effectively induces apoptosis of leukemia cells in CML cell lines and mouse models. Similar strategies can be used to disrupt other fusion genes such as EWS-FLI1 (Ewing sarcoma) and PML-RARA (acute promyelocytic leukemia). However, because these tumors often harbor additional gene mutations and the fusion breakpoints vary among patients, universal design remains challenging.

(3) CRISPR library screening for drug targets

Genome-wide CRISPR knockout libraries enable systematic screening of genes essential for cell proliferation, survival, or drug sensitivity in specific tumor cell backgrounds, thereby rapidly identifying new therapeutic targets. For example, in KRAS-mutant pancreatic cancer cells, CRISPR library screening identified genes involved in synthetic lethality (e.g., KEAP1, STK33), providing candidates for targeted drug development. Although this application is not a direct therapy, it serves as a powerful tool for precision drug discovery and significantly advances precision oncology.

4.1.2 Core Challenges

(1) Tumor heterogeneity and clonal evolution

Solid tumors typically contain multiple subclones with distinct mutational profiles, and tumor cells evolve rapidly under therapeutic pressure. Even if CRISPR completely edits the target in one subclone, other unedited subclones can still lead to relapse. Adding to the complexity, mutational profiles may differ among distinct metastatic lesions within the same patient. To quantify this issue, a simplified model is introduced:

$$P_{elimination} = 1 - \prod_{i=1}^{N} (1 - p_i)$$

where N is the number of detectable subclones within the tumor and p_i is the probability of successful treatment for the i-th subclone. If all subclones have p_i close to 1, the overall elimination probability approaches 1. In practice, however, due to uneven delivery and mutational heterogeneity, some subclones may have very low or even zero p_i, leading to a marked decrease in $P_{elimination}$. For example, if N=10, with eight subclones having p=0.99 and two subclones having p=0.1, the overall success probability is approximately $1-(0.018 \times 0.92) \approx 0.9992$, which still appears high; but if one subclone has p=0, then $P_{elimination}=0$. Tumor heterogeneity thus makes the complete elimination of all cancer cells theoretically extremely difficult.

(2) In vivo delivery bottlenecks

Unlike the highly targetable liver in monogenic diseases or ex vivo editing of HSCs, solid tumors reside in various organs and are surrounded by dense stroma. After systemic administration, the enrichment of CRISPR components in tumors is generally very low. LNPs primarily accumulate in the liver; AAVs, despite having multiple serotypes, are prone to neutralization by pre-existing antibodies and have a limited packaging capacity (<4.7 kb), making it difficult to accommodate both SpCas9 (~4.2 kb) and sgRNA expression cassettes. Local injection (e.g., intratumoral, intracranial) can increase local concentration but is

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not applicable to multiple or metastatic tumors. VLPs and polymeric nanocarriers developed in recent years remain at the preclinical stage.

(3) Safety: off-target effects and genomic instability

In addition to off-target effects, CRISPR editing in cancer cells poses another unique risk: genomic instability. Cancer cells frequently harbor defects in DNA repair pathways, and CRISPR-induced double-strand breaks may be misrepaired, leading to chromosomal translocations, large deletions or insertions, and even activation of latent proto-oncogenes. Furthermore, even single-strand nicking by Cas9 can, in certain contexts, trigger p53-mediated cell cycle arrest, which may instead select for p53-mutated cell clones, increasing the risk of secondary tumors. Therefore, the application of CRISPR in cancer requires extremely cautious risk-benefit assessment.

$$R_{off-target} = k \cdot C_{sgRNA} \cdot t \cdot f_{similarity}$$

where $R_{off-target}$ is the expected number of off-target events per unit time; k is a constant dependent on the Cas9 variant; C_{sgRNA} is the effective intracellular concentration of the sgRNA; t is the duration of editing; and $f_{similarity}$ is the frequency of unintended sequences in the genome that are similar to the target sequence. This formula suggests that restricting sgRNA concentration and exposure time, using high-fidelity Cas9 variants, and optimizing sgRNA design to reduce similarity to off-target sequences are major strategies to reduce off-target risk.

4.2 Long COVID

4.2.1 Overview of Pathological Mechanisms

Long COVID is defined as a syndrome in which symptoms persist for more than 12 weeks after SARS-CoV-2 infection and cannot be explained by an alternative diagnosis. It involves over 200 different symptoms, including fatigue, cognitive impairment ("brain fog"), dyspnea, and autonomic dysfunction. The pathological mechanisms are not yet fully elucidated. Current mainstream hypotheses include: (1) viral persistence: viral RNA or even replication-competent virus persists long-term in extrapulmonary tissues (e.g., gut, nervous system, bone marrow), continuously triggering immune activation; (2) immune dysregulation: post-infection T-cell exhaustion, activation of autoreactive B cells, and sustained elevation of cytokines such as IL-6 and TNF-α; (3) mitochondrial dysfunction leading to abnormal energy metabolism; (4) microvascular endothelial injury and microthrombosis. Notably, different patients may be dominated by different mechanisms, resulting in extremely high heterogeneity.

4.2.2 Application Prospects

Given the complexity of long COVID, direct therapeutic application of CRISPR-Cas9 is still at a very early conceptual stage. However, its potential as a research tool is already evident.

(1) Targeting viral reservoirs

If persistent SARS-CoV-2 reservoirs are confirmed in specific tissues where the viral genome is actively transcribed, CRISPR-Cas9 (for DNA cleavage) or CRISPR-Cas13 (for RNA cleavage) could be considered to destroy the viral genome. Cas13 is particularly suitable for RNA viruses because it does not cleave the host genome, is reversible, and has higher safety. However, delivery to putative reservoirs (e.g., intestinal mucosa, brain tissue) is extremely challenging, and much of the viral RNA may already be integrated into the host genome (although SARS-CoV-2 is an RNA virus, there are reports of retrotransposition integration). Targeting strategies must be highly specific to avoid damaging essential host genes.

(2) Modulating immune responses

For immune dysregulation, CRISPR could be used to edit key functional genes in regulatory T cells (Tregs) to enhance their ability to suppress autoreactive T cells. Another strategy is to knock out pro-inflammatory cytokines (e.g., IFN-γ, TNF-α) or their receptors in effector T cells to curb excessive inflammation. These strategies have shown some potential in preclinical models of autoimmune diseases, but their application to long COVID will first require identification of which immune pathways are critical for restoring homeostasis.

(3) CRISPR screening to identify host susceptibility genes

Using genome-wide CRISPR knockout or activation libraries to screen for host factors that influence persistent symptoms after SARS-CoV-2 infection in human cell lines could provide clues to the molecular basis of long COVID. For example, genes involved in viral persistence, immune evasion, or autoantigen presentation could be identified, and then small molecules or antibody drugs could be developed. This application uses CRISPR as a "discovery tool" rather than a "therapeutic modality" and represents the most realistic and valuable direction at present.

4.2.3 Major Challenges

The prospect of direct therapeutic application of CRISPR in long COVID is distant. At present, its most important and responsible role in long COVID is as a research tool to help elucidate disease mechanisms and discover actionable targets. First, lack of clear targets. Long COVID lacks a clear, actionable genetic target. Unlike the linear logic of "mutation A → disease B" in monogenic diseases, the pathological network of long COVID is highly non-linear, and the molecular phenotype may differ dras-

tically among different patients or even within the same patient over time. Second, pathological heterogeneity. Current diagnostic criteria cannot distinguish between different subtypes of long COVID. A CRISPR therapy designed against the "viral persistence" hypothesis may be completely ineffective—or even harmful—for patients whose dominant mechanism is "autoimmunity". Achieving precision therapy will first require fine-grained stratification of long COVID patients using single-cell multi-omics and spatial transcriptomics. Third, delivery and timing difficulties. If viral reservoirs are located in the central nervous system or deep in the gut, existing delivery systems cannot easily cross the blood-brain barrier or penetrate the mucosa. Moreover, long COVID is a chronic syndrome lasting months or even years, whereas CRISPR editing is permanent. Introducing permanent genomic edits in tissues with a high inflammatory burden could trigger unpredictable long-term immune consequences. Fourth, ethical and risk-benefit assessment. Although long COVID seriously impairs quality of life, it is not typically immediately life-threatening. Permanent genome editing for a non-fatal disease would be subject to extremely stringent ethical review. A more rational approach is to prioritize the development of reversible intervention strategies rather than direct CRISPR use.

4.3 Common Challenges and Future Strategies

The delivery system is a critical ratelimiting step for the clinical translation of CRISPRCas9. For both monogenic and complex diseases, the effective editing dose must reach a tissuespecific threshold to achieve clinical benefit. To quantify this relationship, this review proposes the following model:

$$E_{clinical} = \int_0^T D(t) \bullet R_{editing} dt \geqslant E_{threshold}$$

where $E_{clinical}$ is the cumulative editing dose, $D(t)$ is the rate at which the delivery system delivers CRISPR components to the target cells over time t, $R_{editing}$ is the success rate of a single editing event, and $E_{threshold}$ is the minimum editing dose required to achieve a clinical effect. In monogenic diseases, $E_{threshold}$ is relatively low, and livertargeted LNPs can achieve a high $D(t)$. In solid tumors, by contrast, $E_{threshold}$ is extremely high, while the tumor microenvironment greatly reduces $D(t)$. Future breakthroughs include engineered AAV serotypes with tropism modification, actively targeted LNPs, biomimetic carriers, and viruslike particles.

Detection and elimination of offtarget effects constitute another core challenge. Existing detection technologies include GUIDEseq, DISCOVERseq, and wholegenome sequencing. In the preclinical phase, a combination of methods should be used to screen potential offtarget sites, and periodic monitoring should be performed during clinical trials. Engineering strategies include highfidelity Cas9 variants (eSpCas9, SpCas9HF1), truncated sgRNAs, and base editing or prime editing. In terms of ethics and regulation, germline editing is widely recognized as a "red line". For somatic cell therapy, it is necessary to establish mechanisms for longterm followup, informed consent, and cost accessibility, and to promote equitable global access.

5. Conclusion

CRISPR-Cas9 has achieved landmark clinical success in the treatment of common monogenic diseases. Phase I/II trials represented by SCD, TDT, and ATTR have demonstrated that durable clinical remission with a controllable safety profile can be achieved through ex vivo editing of autologous hematopoietic stem cells or in vivo delivery to the liver via lipid nanoparticles. These outcomes validate the feasibility of gene editing as a curative therapeutic strategy and provide a powerful paradigmatic tool for precision medicine. However, when extending these successes to complex diseases such as cancer and long COVID, the challenges increase exponentially. Cancer confronts tumor heterogeneity, in vivo delivery bottlenecks, and genomic instability, whereas long COVID lacks a defined genetic target, exhibits highly heterogeneous pathological mechanisms, and poses formidable delivery difficulties. These differences necessitate a shift in application logic from "repair" to "engineering modification" and "immunomodulation," along with systematic breakthroughs in delivery systems, off-target detection, and ethical regulation. Looking forward, the most promising directions in the short term remain ex vivo editing of blood cells/immune cells and in vivo editing of the liver. For long COVID, a more realistic positioning of CRISPR is as a research tool to aid mechanistic dissection and target screening. Ultimately, the success of CRISPR-Cas9 in monogenic diseases serves as the prelude to precision medicine, while conquering complex diseases will constitute a longer and more transformative main chapter.

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