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Research Progress of CRISPR-Cas Technology in the Treatment of Monogenic Diseases

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Abstract. CRISPR-Cas technology has undergone three generations of iterative evolution and has become the core strategy for the treatment of monogenic disorders. The first-generation standard nucleases typified by Cas9 and Cas12a rely on double-strand breaks (DSB) to achieve gene disruption and reactivation, which have been successfully translated into clinical practice and led to the approval of the first CRISPR therapy exagamglogene autotemcel (exa-cel) for β-hemoglobinopathies. The second-generation tools including base editors and prime editors realize DSB-free precise DNA modification, providing solutions for point mutations and small fragment lesions, and have entered early clinical trials. The third-generation represented by Cas13 RNA editing and epigenome editing achieves reversible and controllable gene regulation without altering genomic DNA, which is suitable for neurodegenerative and epigenetic monogenic diseases. This review systematically summarizes the developmental logic, core mechanisms, clinical applications, advantages and limitations of the three generations of CRISPR-Cas technology in monogenic disorders, and analyzes the key challenges such as delivery efficiency, long-term safety, and treatment accessibility, as well as future directions including genome writing and AI-driven optimization. It aims to provide a reference for the further clinical translation of CRISPR-based gene therapy for monogenic diseases.

Keywords: CRISPR-Cas, monogenic disorders, gene editing, base editing, prime editing, RNA editing

1. Introduction

1.1. Current status of monogenic genetic diseases and limitations of traditional treatments

Monogenic genetic diseases are hereditary disorders controlled by a pair of alleles and follow Mendelian inheritance patterns. Up to 2024, over 7,000 monogenic diseases are known (Online Mendelian Inheritance in Man (OMIM) database), classified into four types: autosomal dominant genetic diseases (such as Huntington's chorea), autosomal recessive genetic diseases (such as sickle cell anemia), X-linked dominant genetic diseases, and X-linked recessive genetic diseases (such as hemophilia). Their global incidence rate is approximately 1/1000 to 1/2000, affecting millions of people and posing significant public health challenges. These diseases severely impact the quality of life of patients. Patients often undergo a lengthy delayed diagnosis, requiring an average of 5 to 7

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years to obtain a definitive diagnosis [1].The vast majority of monogenic diseases currently lack curative treatment options. Traditional clinical interventions are limited to symptomatic management, lifelong drug maintenance, or organ transplantation, which are costly, burdensome for patients, and do not address the underlying genetic root cause of the disease.Most diseases have the characteristics of early onset, progressive nature, disability, or death, imposing a heavy burden on families and society. Therefore, the development of curative treatment methods is urgent.

1.2. The rise of gene therapy

The concept of gene therapy was first proposed in 1972. In 1990, Anderson's team treated severe combined immunodeficiency (SCID) using retroviral vectors, marking the beginning of clinical gene therapy. Nevertheless, early viral integration caused insertion mutations, failing to meet precise therapeutic requirements. In 2023, Casgevy, a CRISPR-based gene-editing therapy, was approved for sickle cell disease (SCD) and transfusion-dependent beta-thalassemia (TDT), strongly validating the clinical feasibility of gene editing for monogenic disorders [2-4].

1.3. CRISPR-Cas system and treatment of monogenic diseases

Unlike conventional drugs, the CRISPR-Cas system directly repairs pathogenic DNA mutations, offering potential one-time radical cures. Monogenic diseases possess definite genetic causes and clear editing targets, making them ideal candidates for gene editing . To date, CRISPR-Cas technologies have undergone three generations of iteration: the first generation (2012–2015) acts as "gene scissors", the second (2016–2019) as "gene pencils", and the third (2017/2021–present) as "gene switches". Rather than simple replacement, these technologies develop in parallel to satisfy diverse clinical demands, collectively forming a comprehensive technical toolbox for monogenic disease treatment.

1.3.1. First generation: dependent on double-strand breaks (DSB) standard nucleases

Represented by Cas9, the first-generation system induces targeted DSB and relies on NHEJ or HDR pathways to achieve gene knockout or knock-in. Discovered in 2015, Cas12a exhibits unique advantages such as tracrRNA independence, sticky-end cleavage and lower off-target rates [5]. The most successful application is hematopoietic stem cell editing for hemoglobinopathies; Casgevy, approved in 2023, reactivates fetal hemoglobin to permanently cure SCD and TDT through one-time administration [6]. However, DSB-related limitations restrict further application: NHEJ randomness hinders precise point-mutation repair, low HDR efficiency limits non-dividing cell treatment, and p53-associated cytotoxicity impedes editing efficiency [7]. These drawbacks promoted the emergence of second-generation editing tools.

1.3.2. Second generation: precise DNA editing without DSB

To eliminate DSB-associated risks, DSB-free second-generation editing technologies were developed [8]. Base editors (CBE and ABE) enable single-base conversion without donor templates, correcting over 25% of human pathogenic single-nucleotide variations for point-mutation diseases such as cystic fibrosis [9]. Prime editors employ reverse transcriptase and pegRNA to realize all base conversions and small fragment indels, showing superiority in complex mutations including Huntington's chorea and DMD, with ongoing non-human primate verification and upcoming clinical trials [10, 11]. Despite the leap toward precise rewriting, second-generation editing induces

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permanent DNA modification, which is unsuitable for dynamically regulated neurodegenerative disorders.

1.3.3. Third generation: no DNA sequence alteration for regulation

The third generation focuses on reversible transcriptional regulation, particularly suitable for neurodegenerative monogenic diseases requiring transient intervention [12]. The Cas13 system targets mRNA instead of genomic DNA to achieve reversible gene knockdown. As the smallest Cas13 subtype, Cas13d is compatible with AAV in vivo delivery, providing safe reversible treatment for ALS and TTR amyloidosis without permanent genetic alteration [13].

Besides the three generations of core technologies mentioned above, the CRISPR field has developed a variety of advanced editing and regulatory tools, further expanding the therapeutic boundaries for monogenic diseases. Figure 1 systematically illustrates the working mechanisms and functional advantages of these emerging technologies.

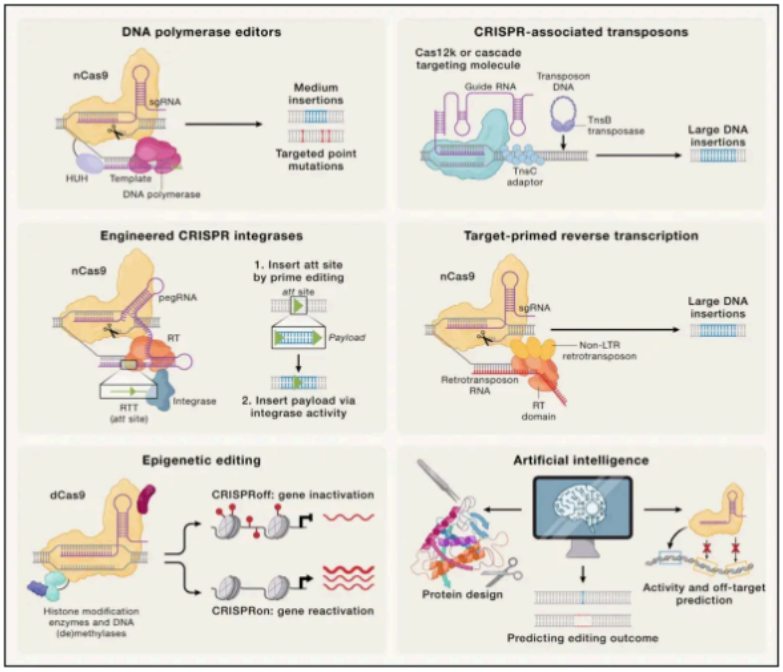

Figure 1. Overview of advanced CRISPR-based genome editing and regulatory technologies

This figure depicts six key emerging CRISPR tools, including DNA polymerase editors, CRISPR-associated transposons, engineered integrases, target-primed reverse transcription, epigenetic editors, and AI-assisted design platforms. These technologies address limitations of conventional CRISPR systems, enabling precise point mutations, large DNA insertions, reversible gene regulation, and improved editing specificity for monogenic disease therapy.

1.4. The purpose and significance of this study

Current reviews usually focus on individual CRISPR branches, while systematic collation and clinical comparison of three-generation editing technologies remain insufficient. This review summarizes the iterative evolution of CRISPR-Cas systems under the guidance of "clinical demand-driven technological upgrading". It comprehensively analyzes the properties, applications, advantages and limitations of Cas9/Cas12/Cas13, base editors and prime editors in monogenic

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disease therapy. This paper further discusses translational challenges and future directions, aiming to provide systematic references for subsequent fundamental research and clinical transformation of gene-editing therapies.

2. Clinical applications of first-generation CRISPR technology in monogenic disorders

2.1. Unmet clinical needs and treatment strategy selection

β-hemoglobinopathies, including sickle cell disease (SCD) and β-thalassemia (TDT), represent a critical unmet need in monogenic disease care. Patients with SCD have significantly shortened lifespans, and recurrent vaso-occlusive crises (VOCs) damage multiple organs and reduce quality of life. Patients with TDT depend on lifelong transfusions and face life-threatening complications from iron overload, including heart failure and endocrine dysfunction. Cardiomyopathy and heart failure remain the leading causes of death in this population [14-17]. Although early gene therapy efforts showed promise, randomly integrated viral vectors carried risks of insertional mutagenesis, and transgene expression was difficult to control precisely. This prompted researchers to pursue gene-editing strategies that enable precise targeting without permanent integration of exogenous sequences. A key biological insight—that endogenous fetal hemoglobin (HbF) can effectively compensate for defective adult hemoglobin, and that BCL11A specifically represses HbF in erythroid cells—provided a precise target for the "disruption to activate" therapeutic paradigm. By targeting the erythroid-specific enhancer of BCL11A, HbF can be safely and reversibly reactivated while preserving other physiological functions of BCL11A.

2.2. Proof of concept: breakthrough in the first patients

Between 2019 and 2021, Frangoul et al. translated this strategy to the clinic. In this study, Cas9 ribonucleoprotein complexes were delivered into patient-derived CD34+ hematopoietic stem/progenitor cells (HSPCs) by electroporation, targeting the +58 site of the BCL11A enhancer. This strategy showed significant efficacy in the first two patients (one with TDT and one with SCD): in vitro editing efficiency reached 80%. After 15 months of follow-up, the TDT patient achieved complete transfusion independence, and the SCD patient completely eliminated VOCs, with HbF distributed in >99% of cells. This breakthrough verified that the ex vivo editing and reinfusion strategy can meet the core clinical needs of single-dose administration and functional cure [6].

In the same period, Esrick et al. used a lentiviral vector-mediated post-transcriptional silencing strategy for BCL11A and verified the effectiveness of an alternative gene regulation pathway in 7 patients with SCD. After a median follow-up of 15 months, HbF levels reached 43.2%, the proportion of F cells exceeded 95%, and VOCs were completely eliminated [18]. The parallel success of these two technical routes reflects the urgent clinical demand for the therapeutic endpoint of HbF reactivation and also indicates the flexibility of technology platform selection.

2.3. Efficacy confirmation: from individual cases to cohort expansion

The success of proof of concept promoted the rapid expansion of clinical scale to meet the clinical regulatory requirements of reproducible efficacy and long-term safety. From 2021 to 2022, pooled data from the CLIMB THAL-111 and CLIMB SCD-121 trials included 75 patients: 42 of 44 TDT patients (95.5%) achieved transfusion independence, and all 31 SCD patients eliminated VOCs, with the longest efficacy lasting 37 months. This stage confirmed the robustness and durability of gene-

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editing efficacy, and its benefits showed reproducible clinical laws, providing key evidence for the transformation from experimental treatment to standard treatment.

2.4. Regulatory approval: Phase III trials and standardized treatment

Based on the above evidence, key Phase III trials established the regulatory basis for exagamglogene autotemcel (exa-cel) in response to the needs of large-scale clinical application [3, 19]. In the CLIMB SCD-121 trial, 44 patients with SCD were treated, and 29 of 31 evaluable patients (93.5%; 98% CI: 77.9%–100%) achieved the primary endpoint of being free of severe VOCs for at least 12 months [3]. In the CLIMB THAL-111 trial, 52 patients with TDT were treated, and 32 of 35 evaluable patients (91.4%; 98.3% CI: 75.7%–100%) achieved transfusion independence for at least 12 months. Patients who reached the primary endpoint maintained transfusion independence for a median of 20.8 months, with a mean weighted total hemoglobin of 13.1 g/dL [19].

In December 2023, the U.S. FDA approved exagamglogene autotemcel as the world's first CRISPR gene-editing therapy, heralded the era of this technology from experimental intervention into standard treatment. This milestone responds to the long-term clinical demand of patients with β-hemoglobinopathies for curative therapies [2].

2.5. Variants of first-generation technology: clinical exploration and need adaptation of Cas12a

The clinical success of first-generation Cas9 technology has also exposed new technical constraints: PAM sequence restrictions (5'-NGG-3') make it difficult to target AT-rich regions, blunt-end cleavage may increase the risk of misligation and chromosomal rearrangement, and the large molecular weight of SpCas9 (~4.3 kb) limits the in vivo delivery efficiency of vectors such as AAV [20]. These practical problems in clinical translation have driven the exploration of alternative nuclease platforms.Cas12a (Cpf1) is regarded as an alternative that can meet more flexible targeting needs due to its unique enzymatic properties: no need for tracrRNA, production of sticky ends after cleavage, trans-cleavage activity, and potentially lower off-target rates [5]. The ability of Cas12a to target AT-rich regions provides new possibilities for sickle cell disease (HBB gene is AT-rich), and its smaller size facilitates AAV delivery to hematopoietic stem cells. In 2023, Editas Medicine launched the first clinical trial of Cas12a (EDIT-301, now reni-cel), using engineered enhanced AsCas12a (enAsCas12a) to edit HSPCs and treat SCD and TDT by disrupting the erythroid enhancer of BCL11A [21]. As of the data reported at the 2023 ASH Annual Meeting in December 2023, 17 patients (11 with SCD and 6 with TDT) had been treated: all SCD patients eliminated VOCs with HbF levels >40%; all TDT patients broke away from transfusion dependence with HbF levels >60%. This study verified the safety and efficacy of Cas12a in humans for the first time, providing an alternative enzymatic platform for first-generation technology.

2.6. Unmet clinical needs and drivers of technical iteration

The success of first-generation CRISPR also reveals unmet clinical needs that drive further technical iteration.First, point mutation correction requires higher precision, as random NHEJ repair cannot meet the standards for diseases such as cystic fibrosis and Duchenne muscular dystrophy. Second, non-dividing cell editing is limited by low HDR efficiency, restricting applications in neurodegenerative and muscular disorders. Third, treatment accessibility is constrained by high costs, ex vivo handling, and reliance on GMP facilities, especially in low– and middle–income

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countries.Together, these needs drive the shift from "disruption to activate" toward precision correction and in vivo editing, forming the core motivation for second- and third-generation CRISPR.

3. Second-generation CRISPR technology for monogenic diseases: optimization and limitations

3.1. Foundation (2016–2019): establishment of the DSB-free paradigm

First-generation CRISPR-Cas9 relies on HDR and NHEJ, which are inefficient and error-prone for correcting point mutations. From 2016 to 2019, base editing and prime editing enabled a paradigm shift from "disruption" to "precise repair" [7, 8].

Base editors (CBE, ABE) achieve single-base conversion without DSBs or donor templates, and can correct approximately 60% of pathogenic point mutations [9]. Prime editing further enables all 12 base conversions and small indels, covering about 89% of known pathogenic mutations [10].

3.2. Optimization (2020–2022): breaking efficiency bottlenecks and expanding monogenic disease models

From 2020 to 2022, technological optimizations greatly promoted the clinical translation of precise editing for monogenic diseases. In 2021, Chen et al. confirmed that the mismatch repair (MMR) pathway limits editing efficiency by repairing pegRNA-induced mismatches, causing low efficiency and indel byproducts [22]. In 2022, Böck et al. achieved 63% liver editing efficiency via dual AAV8-mediated PE2 delivery in FAH-mutated tyrosinemia mice, with complete phenotypic rescue requiring NTBC combination therapy [23]. The same year, ABE delivered by dual AAV9 induced exon skipping in DMD mdx mice, yet the dystrophin recovery rate only reached 3.3–22% (lower than in vitro), with at least 17% recovery required for phenotypic improvement [24]. Collectively, in vivo editing efficiency in primary cells is typically <20%, constrained by tissues and delivery systems. For cystic fibrosis, Geurts et al. corrected CFTR F508del and W1282X mutations at 50% efficiency using SORT LNP in 2020. However, ABE triggered the R1283G bystander variant, requiring triple therapy and revealing precision control challenges [25].

3.3. Translation (2023–2024): clinical applications of monogenic diseases and exposure of core limitations

From 2023 to 2024, monogenic disease therapies based on base editing and prime editing entered clinical trials, with emerging first-in-human data and exposed key technical limitations [26].

For p47phox-deficient chronic granulomatous disease (CGD) caused by the 2-bp deletion of the NCF1 gene, PM359 is an ex vivo prime-edited autologous HSPC therapy. The FDA approved its IND amendment for expanded enrollment in May 2024. Nevertheless, Prime Medicine stopped independent development of the CGD program and sought external partnerships, reflecting the survival disadvantage of precise editing technology in hematopoietic stem and progenitor cells [26]. In hemoglobinopathy research, BEAM-101 uses ABE8e to modify the HBG1/2 promoter region, disrupt BCL11A binding sites and reactivate HbF. Trial data released in December 2024 showed HbF levels above 60% in four SCD patients without vaso-occlusive crises during follow-up. The therapy obtained FDA RMAT designation, with enrollment completion planned for mid-2025 and BLA submission by late 2026 [27]. As the first prime-editing therapy to directly correct HBB point

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mutation, GPH101 completed the first patient dosing in the CEDAR trial in August 2022. The trial was voluntarily suspended after the first patient developed persistent pancytopenia in January 2023. This event revealed the survival disadvantage of precise editing in hematopoietic stem and progenitor cells [28].

3.4. Limitations of second-generation technology and transition to the third generation

Although second-generation editing avoids DSBs, inherent drawbacks limit its application in monogenic diseases and drive third-generation technological iteration. Its editing efficiency remains low and tissue-dependent: DMD only achieves 3.3%–22% correction [24], cystic fibrosis lung-targeted editing stays confined to organoid models [25], and the blood–brain barrier blocks neurological disease treatment, with primary cell efficiency often under 20%. Bystander effects compromise precision; ABE editing at the CFTR W1282X site may trigger the R1283G variant [25], while prime editing risks off-target sequence alterations, promoting optimized deaminases and ultra-precise editing design. AAV immunogenicity and poor dual-AAV co-transduction also bring safety limitations [29], advancing non-viral vectors including ionizable lipids, VLPs and extracellular vesicles. Constrained by NGG PAM sequences, nearly half of monogenic mutations are inaccessible, facilitating the development of PAM-less SpRY and Cas12a tools [30]. Irreversible DNA modification fails to adapt to temporary intervention needs, driving inducible editors, CRISPR-off systems [12] and reversible epigenome editing. Meanwhile, base editing enables the construction of universal CAR-T products, yielding promising efficacy in pediatric leukemia and supporting accessible standardized cell therapy [31].

4. Third-generation CRISPR: epigenetic & RNA editing for monogenic disorders

4.1. Core principle and classification of third-generation editing

Third-generation CRISPR technology mainly includes two core types: RNA editing and epigenetic editing. The biggest advantage of this generation of technology is that it does not alter the underlying DNA sequence of the genome, which avoids the long-term safety risks caused by permanent DNA modification and provides a safer therapeutic alternative for treating irreversible neuronal monogenic diseases and pediatric genetic disorders [12, 13].

Among them, Cas13-mediated RNA editing achieves reversible gene silencing and post-transcriptional regulation by targeting messenger RNA (mRNA), which is particularly suitable for temporary intervention of dominant genetic mutations and diseases that require dynamic regulation [13]. Epigenetic editing, on the other hand, regulates gene expression through DNA methylation and histone modification, which can achieve long-term therapeutic effects without causing any damage to the genome [12].

4.2. Advantages and application prospects of third-generation technology

Epigenome editing marks the core theoretical breakthrough of third-generation technology, enabling sustained yet reversible gene regulation via chromatin modulation without DNA sequence alteration. Developed in 2021, CRISPRoff fuses dCas9 with repressive domains to deposit inhibitory epigenetic marks at target promoters [12]. Its regulatory effect can be stably inherited during cell division, with gene activity restorable by CRISPRon-mediated demethylation. Overcoming the limitation of CpG-island-dependent silencing, this system effectively represses pathogenic Tau and

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huntingtin genes throughout neuronal differentiation [12], providing a reversible intervention approach for chronic monogenic neurodegenerative diseases and eliminating irreversible gene editing risks.

Epigenome editing still confronts prominent challenges. Epigenetic memory stability differs across tissues, and certain disease-related genes show silencing resistance due to chromatin features. Collateral epigenetic effects may spread to adjacent genes or transmit to germ cells [12]. Though epigenome editing does not change DNA sequences, potential transgenerational epigenetic inheritance demands rigorous safety and ethical assessment, especially in embryonic and germline editing for monogenic disease treatment.

4.3. Clinical exploration of third-generation technology

At present, third-generation CRISPR editing is still in the preclinical research and early clinical exploration stage. Cas13-based RNA editing has been applied to the research of neurodegenerative monogenic diseases, achieving effective suppression of abnormal gene expression without changing genomic DNA [13]. Epigenetic editing can reactivate silenced functional genes by modifying epigenetic markers, providing a new therapeutic route for recessive genetic disorders caused by gene downregulation [12]. Compared with gene knockout and base correction strategies, this reversible regulation mode reduces the risk of irreversible genome mutation and has higher clinical safety margin.

5. Current challenges and future perspectives for monogenic disease therapy

5.1. Current major challenges

Despite promising advances in CRISPR technology for monogenic disorders, its clinical translation still faces prominent hurdles. Limited tissue-specific delivery restricts clinical application: DMD shows a low muscle editing efficiency of 3.3–22% [24], lung-targeted editing for cystic fibrosis remains confined to organoid models [25], and the blood–brain barrier greatly impedes intervention for neuronal monogenic diseases. Clinical setbacks of GPH101 and the suspension of the PM359 program reflect the survival disadvantage of precision-edited hematopoietic stem and progenitor cells, revealing distinct safety profiles between prime editing and NHEJ-free base editing strategies. Mitochondrial monogenic disease therapy is constrained by limited editing tools, with only C・G-to-T・A conversion currently achievable for mtDNA mutations [32]. Moreover, early-life intervention requires lifelong safety monitoring, concerning transgenerational epigenetic inheritance risks [12], cumulative immunogenicity, and imperfect regulatory standards for reversible editing technologies.

5.2. Future development perspectives

Future development focuses on three directions. AI-designed Cas proteins and twinPE enable large-fragment genome manipulation for complex pathogenic mutations [33, 34]. Advanced mammalian genome writing further achieves ultra-long locus integration and structural variation repair, compensating for the limitations of traditional editing tools [35]. Additionally, base-edited universal CAR7-T cells improve the commercialization potential of standardized cell therapy [31]. Combined with optimized in vivo delivery and high-throughput preparation systems, these technologies reduce GMP dependence and expand global therapeutic accessibility [35]. Furthermore, generative AI

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cooperates with genome writing to construct a closed-loop design–build–verify–iterate system, accelerating pathological mechanism analysis and individualized treatment optimization. This AI-driven strategy promotes the technical shift from simple mutation repair to de novo genomic design for intractable monogenic diseases [35].

6. Conclusion

Driven by clinical demands, CRISPR-Cas technology has evolved iteratively for monogenic disease treatment. The first-generation Cas9/Cas12a induces DNA double-strand breaks to disrupt genes and cures hematological monogenic disorders. Second-generation base and prime editing avoid double-strand breaks for precise point-mutation correction. The third-generation platforms support reversible modulation and large-fragment reconstruction, targeting complex and neurodegenerative monogenic diseases. These three generations complement rather than replace one another for differentiated clinical scenarios. Novel tools including PE7, AI-engineered Cas proteins and twinPE facilitate the construction of intelligent editing systems. Nevertheless, tissue-targeted delivery barriers, uncertain long-term safety, poor accessibility and imperfect ethical constraints remain major challenges. Further optimization of in vivo editing efficiency and automated production will accelerate the clinical popularization of CRISPR technology and promote the therapeutic paradigm transformation of monogenic diseases.

References

[1] Cabré-Romans, J.-J., & Cuella-Martín, R. (2025). CRISPR-dependent base editing as a therapeutic strategy for rare monogenic disorders. Frontiers in Genome Editing, 6, 1553590. https: //doi.org/10.3389/fgeed.2025.1553590
[2] Food and Drug Administration. (2023). FDA approves first gene therapies to treat patients with sickle cell disease. U.S. Food and Drug Administration. https: //www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease
[3] Medicines and Healthcare products Regulatory Agency. (2023). MHRA authorises world first gene therapy that aims to cure sickle cell disease and transfusion-dependent thalassaemia. GOV.UK. https: //www.gov.uk/government/news/mhra-authorises-world-first-gene-therapy-that-aims-to-cure-sickle-cell-disease-and-transfusion-dependent-thalassemia
[4] Frangoul, H., Locatelli, F., Cappellini, M. D., et al. (2024). CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. New England Journal of Medicine, 390(3), 251–263. https: //doi.org/10.1056/NEJMoa2309676
[5] Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., et al. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell, 163(3), 759–771. https: //doi.org/10.1016/j.cell.2015.09.038
[6] Frangoul, H., Altshuler, D., Cappellini, M. D., et al. (2021). CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. New England Journal of Medicine, 384(3), 252–260. https: //doi.org/10.1056/NEJMoa2031054
[7] Komor, A. C., Kim, Y. B., Packer, M. S., et al. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603), 420–424. https: //doi.org/10.1038/nature17946
[8] Gaudelli, N. M., Komor, A. C., Rees, H. A., et al. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature, 551(7681), 464–471. https: //doi.org/10.1038/nature24644
[9] Kantor, A., McClements, M. E., & MacLaren, R. E. (2020). CRISPR-Cas9 DNA base-editing and prime-editing. International Journal of Molecular Sciences, 21(17), 6240. https: //doi.org/10.3390/ijms21176240
[10] Anzalone, A. V., Randolph, P. B., Davis, J. R., et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149–157. https: //doi.org/10.1038/s41586-019-1711-4
[11] Murray, J. B., Harrison, P. T., & Scholefield, J. (2024). Prime editing: Therapeutic advances and mechanistic insights. Gene Therapy, 32(1–2), 83–92. https: //pmc.ncbi.nlm.nih.gov/articles/PMC11946880/
[12] Nuñez, J. K., Chen, J., Pommier, G. C., et al. (2021). Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. Cell, 184(9), 2503–2519.e17. https: //doi.org/10.1016/j.cell.2021.03.025
[13] Cox, D. B. T., Gootenberg, J. S., Abudayyeh, O. A., et al. (2017). RNA editing with CRISPR-Cas13. Science, 358(6366), 1019–1027. https: //doi.org/10.1126/science.aaq0180

DOI: 10.54254/2753-8818/2026.AU35138

[14] Olivieri, N. F., Nathan, D. G., MacMillan, J. H., et al. (1994). Survival in medically treated patients with homozygous beta-thalassemia. New England Journal of Medicine, 331(9), 574–578. https: //doi.org/10.1056/NEJM199409013310903
[15] Platt, O. S., Brambilla, D. J., Rosse, W. F., et al. (1994). Mortality in sickle cell disease. Life expectancy and risk factors for early death. New England Journal of Medicine, 330(23), 1639–1644. https: //doi.org/10.1056/NEJM199406093302303
[16] Platt, O. S., Thorington, B. D., Brambilla, D. J., et al. (1991). Pain in sickle cell disease. Rates and risk factors. New England Journal of Medicine, 325(1), 11–16. https: //doi.org/10.1056/NEJM199107043250103
[17] Brittenham, G. M. (1993). Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. American Journal of Hematology, 42(1), 81–85. https: //doi.org/10.1002/ajh.2830420115
[18] Esrick, E. B., Lehmann, L. E., Biffi, A., et al. (2021). Post-transcriptional genetic silencing of BCL11A to treat sickle cell disease. New England Journal of Medicine, 384(3), 205–215. https: //doi.org/10.1056/NEJMoa2029392
[19] Locatelli, F., Lang, P., Wall, D., et al. (2024). Exagamglogene autotemcel for transfusion-dependent β-thalassemia. New England Journal of Medicine, 390(18), 1663–1676. https: //doi.org/10.1056/NEJMoa2309673
[20] Ran, F. A., Cong, L., Yan, W. X., et al. (2015). In vivo genome editing using Staphylococcus aureus Cas9. Nature, 520(7546), 186–191. https: //doi.org/10.1038/nature14299
[21] Editas Medicine. (2023). Editas medicine announces new EDIT-301 safety and efficacy data. Editas Medicine. https: //ir.editasmedicine.com/news-releases/news-release-details/editas-medicine-announces-new-edit-301-safety-and-efficacy-data
[22] Chen, P. J., Hussmann, J. A., Yan, J., et al. (2021). Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. Cell, 184(22), 5635–5652.e29. https: //doi.org/10.1016/j.cell.2021.09.018
[23] Böck, D., Yu, J., Biermeier, M., Börner, K., Fakhiri, J., Hagedorn, C., Delic, D., Schmeer, M., Schleef, M., Cathomen, T., Müller, A. M., & Grimm, D. (2022). In vivo prime editing of a metabolic liver disease in mice. Science Translational Medicine, 14(636), eabl9238. https: //doi.org/10.1126/scitranslmed.abl9238
[24] Chemello, F., Chai, A. C., Li, H., Rodriguez-Caycedo, C., Sanchez-Ortiz, E., Atmanli, A., Davie, J. K., Bassel-Duby, R., & Olson, E. N. (2021). Precise correction of Duchenne muscular dystrophy exon deletion mutations by base and prime editing. Science Advances, 7(18), eabg4910. https: //doi.org/10.1126/sciadv.abg4910
[25] Geurts, M. H., de Poel, E., Amatngalim, G. D., Oka, R., Meijers, F. M., Kruisselbrink, E., van Mourik, P., Vonk, A. M., de Winter-de Groot, K. M., Janssens, H. M., van der Ent, C. K., Beekman, J. M., & Clevers, H. (2020). Evaluating CRISPR-based base editing to correct the two most common cystic fibrosis-causing mutations in intestinal organoids. Cell Stem Cell, 26(4), 503–510.e7. https: //doi.org/10.1016/j.stem.2020.01.019
[26] Prime Medicine (2024) Strategic restructuring and updates on PM359 CGD program. Prime Medicine. https: //investors.primemedicine.com
[27] Beam Therapeutics (2024) BEAM-101 mechanism and clinical trial overview. Beam Therapeutics.
[28] ClinicalTrials.gov (2023) NCT04819841: CEDAR Trial of GPH101 for Severe Sickle Cell Disease. ClinicalTrials.gov. https: //clinicaltrials.gov/study/NCT04819841
[29] Nelson, J. W., Randolph, P. B., Shen, S. P., Everette, K. A., Chen, P. J., Anzalone, A. V., An, M., Newby, G. A., Chen, K., Hsu, A., & Liu, D. R. (2022). Engineered pegRNAs improve prime editing efficiency. Nature Biotechnology, 40(3), 402–410. https: //doi.org/10.1038/s41587-021-01118-9
[30] Walton, R. T., Christie, K. A., Whittaker, M. N., & Kleinstiver, B. P. (2020). Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science, 368(6488), 290–296. https: //doi.org/10.1126/science.aba8853
[31] Chiesa, R., Georgiadis, C., Syed, F., Zhan, H., Ciocarlie, O., Gkazi, A. S., Adams, S., Kazi, S., Yeung, J., Elvers, R., Al-Janazi, H., Faulkner, M., Mahmood, S., Appleby, J., Bloor, A., Kinnon, C., Qasim, W., & Amrolia, P. (2023). Base-edited CAR7 T cells for relapsed T-cell acute lymphoblastic leukemia. New England Journal of Medicine, 389(10), 899–910. https: //doi.org/10.1056/NEJMoa2300709
[32] Mok, B. Y., de Moraes, M. H., Zeng, J., Bosch, D. E., Kotrys, A. V., Raguram, A., Hsu, F., Radey, M. C., Peterson, S. B., Mootha, V. K., & Liu, D. R. (2020). A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature, 583(7817), 631–637. https: //doi.org/10.1038/s41586-020-2477-4
[33] Anzalone, A. V., Gao, X. D., Podracky, C. J., Nelson, A. T., Koblan, L. W., Raguram, A., Levy, J. M., Mercer, J. A. M., & Liu, D. R. (2022). Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. Nature Biotechnology, 40(5), 731–740. https: //doi.org/10.1038/s41587-021-01133-w
[34] Ruffolo, J. A., Nayfach, S., Gallagher, J., Bhatnagar, A., Keskin, G., Urchueguía, A., Dinculescu, A., Filshtein, T., Fannjiang, C., Shu, T., Truong, T., Biccichetti, M., Xu, K., Pacesa, M., Fuehrer, R., Shapiro, R. S., Montoya, G., Cameron, P., Viswanathan, R., ... Madani, A. (2025). Design of highly functional genome editors by modelling the universe of CRISPR–Cas sequences. Nature, 641(8063), 518–525. https: //doi.org/10.1038/s41586-025-09298-z

DOI: 10.54254/2753-8818/2026.AU35138

[35] Pinglay, S., Atwater, J. T., Brosh, R., Shendure, J., Maurano, M. T., & Boeke, J. D. (2026). Mammalian genome writing: Unlocking new length scales for genome engineering. Cell, 189(1), 355–374. https://doi.org/10.1016/j.cell.2025.12.034