Global Journal of Research in
Biology and Pharmacy
Journal homepage: https://gsjournals.com/gjrbp/
ISSN: 2980-4221 (Online)

(REVIEW ARTICLE)

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CRISPR-CAS9 AND BASE EDITING: RECENT CLINICAL PROGRESS AND DELIVERY CHALLENGES IN GENE THERAPY

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Global Journal of Research in Biology and Pharmacy, 2023, 02(02), 001–004

Publication history: Received on 08 March 2023; revised on 20 April 2023; accepted on 24 April 2023

Article DOI: https://doi.org/10.58175/gjrbp.2023.2.2.0031

ABSTRACT

CRISPR-Cas9 genome editing has progressed rapidly from laboratory tool to clinical intervention, offering the possibility of durable correction of genetic diseases. Ex vivo editing of hematopoietic stem cells has produced promising results in sickle cell disease and β-thalassemia, while in vivo editing of the transthyretin gene has demonstrated the feasibility of systemic delivery using lipid nanoparticles. Base editing, which enables precise single-nucleotide conversions without introducing double-strand breaks, has emerged as a complementary approach with potential advantages in safety and precision. This review examines recent clinical progress in CRISPR-Cas9 and base editing therapies through early 2023, with a focus on the underlying mechanisms, key clinical data, and persistent delivery challenges. Ex vivo applications are contrasted with in vivo strategies, and the limitations of adeno-associated virus vectors, lipid nanoparticles, and other delivery systems are critically discussed. Off-target editing, immunogenicity, and the need for long-term safety data remain central concerns. Future directions include improved delivery vehicles, prime editing, and multiplexed correction strategies. The evidence indicates that CRISPR-based therapies are entering a decisive phase, but substantial technical and regulatory hurdles must be addressed before broad clinical application.

Keywords: CRISPR-Cas9; Base Editing; Gene Therapy; Lipid Nanoparticles; Adeno-Associated Virus; Off-Target effects

1 INTRODUCTION

The discovery and adaptation of CRISPR-Cas9 as a programmable gene editing tool transformed molecular biology and opened new therapeutic possibilities. By directing the Cas9 nuclease to a specific genomic locus with a guide RNA, researchers can create targeted double-strand breaks that are repaired by non-homologous end joining or homology-directed repair. This enables gene disruption, correction, or insertion. However, double-strand breaks can produce unwanted insertions, deletions, and chromosomal rearrangements. Base editing offers an alternative: catalytically impaired Cas9 fused to a deaminase enzyme can convert one base pair to another without a double-strand break, reducing the risk of large deletions.

Clinical development of CRISPR-Cas9 therapies accelerated in the late 2010s and early 2020s. Ex vivo approaches, in which cells are removed, edited, and reinfused, have led to transformative results for hemoglobinopathies. In vivo approaches, in which editing machinery is delivered directly to target organs, have shown proof of concept in transthyretin amyloidosis. Base editing has reached early clinical trials, with initial patients treated for

hypercholesterolemia and sickle cell disease. Despite this progress, delivery remains the central bottleneck: efficient, specific, and safe delivery of CRISPR components to target cells is required for broader application.

This review critically evaluates recent clinical progress and delivery challenges in CRISPR-Cas9 and base editing gene therapy, emphasizing literature published through early 2023.

2 CRISPR-CAS9 AND BASE EDITING: MECHANISMS

2.1 CRISPR-Cas9

Streptococcus pyogenes Cas9 is an RNA-guided endonuclease. The guide RNA contains a spacer sequence complementary to the target DNA, followed by a protospacer adjacent motif. Upon binding, Cas9 undergoes conformational changes and cleaves both DNA strands, generating a double-strand break. Repair by non-homologous end joining frequently disrupts the target gene through insertions or deletions, while homology-directed repair can introduce precise changes if a donor template is provided. Non-homologous end joining is active in most cell types, whereas homology-directed repair is largely restricted to dividing cells, limiting precise correction in non-dividing tissues.

2.2 Base Editing

Base editors consist of a nickase Cas9 fused to a deaminase. Cytosine base editors convert C•G to T•A, and adenine base editors convert A•T to G•C. A uracil glycosylase inhibitor is included in cytosine base editors to protect the intermediate. Base editing does not require a double-strand break or donor template and is therefore well suited for correcting point mutations in non-dividing cells. However, base editors can generate bystander edits within the editing window and can also cause off-target RNA or DNA edits. Continuous optimization of base editors has improved their fidelity and narrowed the editing window.

3 CLINICAL PROGRESS IN CRISPR-CAS9

3.1 Ex Vivo Editing for Hemoglobinopathies

Sickle cell disease and β-thalassemia are caused by mutations in the β-globin gene. Ex vivo editing of autologous hematopoietic stem and progenitor cells can reactivate fetal hemoglobin, which compensates for defective adult hemoglobin. The most advanced candidate, CTX001, disrupts the erythroid-specific enhancer of BCL11A, a repressor of γ-globin expression. In a landmark 2021 study, Frangoul and colleagues reported that CTX001 eliminated vaso-occlusive crises in sickle cell disease and reduced transfusion requirements in β-thalassemia. These results demonstrated durable engraftment of edited cells and meaningful clinical benefit.

3.2 In Vivo Editing for Transthyretin Amyloidosis

Transthyretin amyloidosis is caused by deposition of misfolded transthyretin protein. NTLA-2001 uses lipid nanoparticles to deliver mRNA encoding Cas9 and a guide RNA targeting the transthyretin gene in hepatocytes. Gillmore et al. reported in 2021 that a single infusion produced dose-dependent reductions in serum transthyretin, with the highest dose achieving over 90% reduction. This was the first demonstration of in vivo CRISPR-Cas9 editing in humans and established LNP-mRNA as a viable delivery system for liver-directed gene editing.

3.3 Other In Vivo Programs

Additional in vivo CRISPR programs include EDIT-101 for Leber congenital amaurosis type 10, delivered by adeno-associated virus to the retina. Early results suggested safety and possible efficacy, though the program was later paused due to limited benefit. Intellia's NTLA-2002 for hereditary angioedema targets the KLKB1 gene in the liver. Phase 1 data showed reductions in plasma kallikrein and attacks. These programs illustrate the expanding scope of in vivo editing.

4 BASE EDITING: CLINICAL PROGRESS

The first clinical base editing therapies entered trials around 2022. VERVE-101 uses an adenine base editor delivered by lipid nanoparticles to inactivate PCSK9 in the liver, lowering LDL cholesterol in heterozygous familial hypercholesterolemia. Preclinical studies in non-human primates demonstrated durable reductions in PCSK9 and LDL cholesterol. Beam Therapeutics initiated BEAM-101 for sickle cell disease, using a base editor to install the hereditary persistence of fetal hemoglobin mutation in hematopoietic stem cells. Additional programs target alpha-1 antitrypsin deficiency and glycogen storage disease. Although clinical data through early 2023 were limited, base editing offers potential advantages in avoiding double-strand breaks and reducing the risk of large deletions.

5 DELIVERY CHALLENGES

5.1 Ex Vivo Delivery

Ex vivo editing avoids many delivery barriers because cells are manipulated outside the body. However, efficient electroporation of mRNA encoding Cas9 and guide RNA must be balanced against cellular toxicity. The requirement for myeloablative conditioning before reinfusion is a significant burden. Additionally, editing efficiencies in long-term repopulating stem cells must be sufficient for durable benefit.

5.2 In Vivo Delivery: Adeno-Associated Virus

Adeno-associated virus vectors are commonly used for in vivo delivery of gene editing components. They provide high transduction efficiency in many tissues and are relatively safe. However, AAV has a limited packaging capacity (~4.7 kb), which is problematic for SpCas9 (~4.2 kb) plus regulatory elements. Smaller Cas9 orthologs, such as SaCas9, can be packaged but may have different PAM requirements. AAV-delivered Cas9 can be expressed for months, increasing the risk of off-target edits and immune responses. Pre-existing anti-AAV antibodies can also limit transduction.

5.3 In Vivo Delivery: Lipid Nanoparticles

Lipid nanoparticles are the delivery system used for NTLA-2001 and mRNA-based therapies. They preferentially deliver cargo to the liver after intravenous administration, which is advantageous for liver-targeted diseases but limits applications in other organs. LNP-mRNA provides transient Cas9 expression, reducing off-target risk. However, extrahepatic delivery requires novel formulations or targeting ligands. LNP-related infusion reactions and immunogenicity are manageable but require monitoring.

5.4 Off-Target Editing and Genotoxicity

CRISPR-Cas9 can cleave at sites with partial complementarity to the guide RNA. Off-target edits can disrupt tumor suppressor genes or cause chromosomal rearrangements. Base editors may also produce off-target deamination. Detection methods such as Digenome-seq, GUIDE-seq, and DISCOVER-seq have improved sensitivity. Clinical candidates undergo extensive off-target analysis, but the long-term consequences of rare off-target events remain uncertain.

5.5 Immunogenicity

Cas9 is a bacterial protein and may elicit immune responses. Many individuals have pre-existing antibodies and T cells against Cas9 orthologs. AAV vectors can also induce immune responses. Transient delivery using LNP-mRNA may reduce adaptive immune activation, but innate immune stimulation from RNA and lipids must be managed.

6 FUTURE PERSPECTIVES

Several strategies are being developed to improve delivery and reduce off-target effects. Prime editing, which uses a Cas9 nickase fused to a reverse transcriptase and a prime editing guide RNA, can install small insertions, deletions, and all 12 base substitutions without a double-strand break or donor template. Although prime editing is currently less efficient than base editing, it offers greater versatility. Improved lipid nanoparticles with targeting ligands may enable delivery to the lung, spleen, and other organs. Non-viral delivery of ribonucleoprotein complexes can reduce immunogenicity and improve specificity. Ex vivo editing may benefit from milder conditioning regimens and safer gene editing tools.

Regulatory frameworks will need to address the unique safety concerns of genome editing, including long-term off-target effects and germline transmission. Patient registries and long-term follow-up studies are essential. The convergence of CRISPR, base editing, and prime editing with advanced delivery systems promises to expand the treatable disease spectrum, but cautious clinical translation is warranted.

7 CONCLUSION

CRISPR-Cas9 and base editing have entered clinical practice, with ex vivo editing for hemoglobinopathies and in vivo editing for transthyretin amyloidosis demonstrating proof of concept. Base editing offers a potentially safer alternative for point mutation correction, and early clinical trials are underway. Delivery remains the primary obstacle: AAV vectors are limited by packaging size and immunogenicity, while lipid nanoparticles are largely confined to the liver. Off-target editing, immunogenicity, and the need for long-term safety monitoring require continued attention. Prime editing and next-generation delivery systems may overcome current limitations. The field is at a critical juncture, with the potential to cure previously untreatable genetic diseases, but rigorous evaluation and ethical oversight are imperative.

REFERENCES

[1] Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–821.

[2] Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819–823.

[3] Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823–826.

[4] Komor AC, Kim YB, Packer MS, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533(7603):420–424.

[5] Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature. 2017;551(7681):464–471.

[6] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149–157.

[7] Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med. 2021;384(3):252–260.

[8] Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med. 2021;385(6):493–502.

[9] Rothgangl T, Dennis MK, Lin PJC, et al. In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol. Nat Biotechnol. 2021;39(8):949–957.

[10] Musunuru K, Chadwick AC, Mizoguchi T, et al. In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature. 2021;593(7859):429–434.

[11] Koblan LW, Erdos MR, Wilson C, et al. In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. Nature. 2021;589(7843):608–614.

[12] Tsai SQ, Zheng Z, Nguyen NT, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat Biotechnol. 2015;33(2):187–197.

[13] Wienert B, Wyman SK, Richardson CD, et al. Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq. Science. 2019;364(6437):286–289.

[14] Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat Med. 2019;25(2):249–254.

[15] Yin H, Song CQ, Dorkin JR, et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol. 2016;34(3):328–333.

[16] Finn JD, Smith AR, Patel MC, et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell Rep. 2018;22(9):2227–2235.

[17] Zuris JA, Thompson DB, Shu Y, et al. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. Nat Biotechnol. 2015;33(1):73–80.

[18] Doudna JA. The promise and challenge of therapeutic genome editing. Nature. 2020;578(7794):229–236.

[19] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018;19(12):770–788.

[20] Wang D, Zhang F, Gao G. CRISPR-based therapeutic genome editing: strategies and in vivo delivery by AAV vectors. Cell. 2020;181(1):136–150.