IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Crispr-Casas Therapeutic Tool: Translational Perspectives From Preclinical Studies To Clinical Trials.

1Rutuja Vilas Sahajrao, 2Sanchit Ramrao Magar, 3Snehal Anil Bhosle, 4Tarnnum Sameer Shaikh

1Student , 2Student , 3Student, 4Student

1Yash Institute Of Pharmacy ,

2Yash Institute Of Pharmacy ,

3Yash Institute Of Pharmacy ,

4Yash Institute Of Pharmacy

Abstract: Introduction- The CRISPR-Cas9 system has revolutionised genome editing by enabling efficient, programmable, and cost-effective modifications of DNA across diverse organisms. Since its adaptation from bacterial adaptive immunity, CRISPR-Cas9 has rapidly transitioned from preclinical models to early human clinical trials, establishing itself as a transformative platform in modern therapeutics. In vitro and in vivo studies have demonstrated its potential to correct disease-causing mutations, model complex pathologies, and accelerate drug discovery. **Methodology**- Clinical applications are already underway in blood disorders such as sickle cell disease and β-thalassemia, where the FDA-approved therapy exagamglogene autotemcel (CASGEVY™) represents the first CRISPR-based treatment. Additional trials, including EDIT-101 for Leber congenital amaurosis and NTLA-2001 for transthyretin amyloidosis, underscore its versatility across genetic and metabolic diseases. CRISPR is also being explored in oncology, infectious diseases, and rare disorders, while next-generation systems such as Cas12, Cas13, base and prime editors, and epigenome regulators are broadening its capabilities. Despite remarkable progress, challenges remain regarding off-target effects, delivery efficiency, immune responses, long-term safety, and ethical concerns, particularly in the context of germline editing. **Conclusion** -Advances in delivery platforms, high-fidelity nucleases, and computational tools are addressing these barriers, while integration

with AI, nanotechnology, and synthetic biology promise to expand the precision and applicability of genome editing. In short, CRISPR-Cas9 is at the vanguard of genetic medicine as a tool for both study and treatment. Its clinical translation offers the unprecedented potential for one-time, curative therapies, but realising this promise will require not only technical refinement but also equitable access, global governance, and ethical responsibility.

Keywords:

- **CRISPR-Cas9 genome editing**
- **Therapeutic gene editing strategies**
- **Clinical genome editing applications**
- **Base and prime editing**
- **Epigenome and transcriptome editing**
- **Gene therapy delivery systems**
- **Off-target editing effects**
- **Sickle cell disease therapy**
- **β-thalassemia gene therapy**
- **Cancer immunotherapy using CRISPR**
- **Rare genetic disease treatment**
- **Personalised precision medicine approaches**

1)Introduction:

A) Brief Overview of Genome Editing Technologies: Genome editing technologies enable precise modifications of DNA sequences in living organisms, revolutionising both basic research and therapeutic applications. Early genome editing tools included **zinc finger nucleases (ZFNs)** and **transcription activator-like effector nucleases (TALENs)**, which rely on engineered DNA-binding proteins fused to nucleases to create site-specific double-strand breaks (Urnov et al., 2010; Joung & Sander, 2013). While powerful, these tools were labour-intensive, expensive, and technically complex to design for each new target. The emergence of CRISPR-Cas systems, which involve Clustered Regularly Interspaced Short Palindromic Repeats, represented a major advancement, as these technologies utilise straightforward RNA-guided approaches for identifying DNA, rendering them more attainable, expandable, and adaptable than previous platforms. (Jinek et al., 2012; Cong et al., 2013).

B) Discovery and Evolution of CRISPR-Cas Systems:

The CRISPR system was initially identified in bacteria and archaea as a component of their adaptive immune response to combat invading viruses and plasmids (BarrangIn 2012, Jinek and colleagues showed that the Cas9 nuclease from Streptococcus pyogenes could be programmed with a single guide RNA (sgRNA) to target and cut specific DNA sequences, opening the door for genome editing in eukaryotic systems (Jinek et al., 2012). Soon after, Cong et al. (2013) and Mali et al. (2013) independently demonstrated the use of CRISPR-Cas9 in mammalian cells. Since then, innovations such as high-fidelity Cas9 variants, base editors, and prime editing have improved its accuracy and therapeutic potential (Komor et al., 2016; Anzalone et al., 2019).

C)Importance of CRISPR-Cas9 in Biomedical Research: CRISPR-Cas9 has rapidly become a cornerstone in biomedical research due to its **simplicity, versatility, and efficiency**. It has been widely employed in functional genomics to identify gene–disease associations, validate drug targets, and create disease models in vitro and in vivo (Shalem et al., 2014; Wang et al., 2014). Importantly, it has accelerated translational research, offering therapeutic opportunities for a range of diseases, including cancer, infectious diseases, blood disorders, and rare genetic conditions (Doudna & Charpentier, 2014). Unlike earlier genome editing platforms, CRISPR-Cas9 allows multiplex editing and can be adapted for epigenome regulation, transcriptome editing, and live-cell imaging, highlighting its broad biomedical utility.

D)Scope of the Review: This review offers a translational outlook on CRISPR-Cas9 as a therapeutic instrument, connecting preclinical findings with current clinical uses. It will outline the mechanistic foundation of CRISPR-Cas9, emphasise preclinical research in disease models, address improvements in delivery mechanisms, and assess the existing advancements in clinical trials. Additionally, it will investigate the

E) challenges and limitations, such as off-target effects, delivery hurdles, and ethical concerns, while considering future directions in precision medicine. By integrating insights from both preclinical and clinical research, this review highlights the transformative potential of CRISPR-Cas9 in advancing the treatment of human diseases.

2. Mechanistic Insights into CRISPR-Cas9: The Cas9 endonuclease is the central effector protein in the CRISPR-Cas9 genome editing system, functioning as an RNA-guided DNA endonuclease that creates site-specific double-strand breaks (DSBs). In terms of structure, Cas9 is composed of two main lobes: the Recognition (REC) lobe and the Nuclease (NUC) lobe, along with a domain that interacts with the PAM (Jinek et al., 2012; Nishimasu et al., 2014). The REC lobe binds to the single-guide RNA (sgRNA), facilitating the formation of the Cas9–sgRNA ribonucleoprotein complex and enabling recognition of the target DNA sequence.

The NUC lobe comprises two catalytic regions: the HNH domain, responsible for cutting the DNA strand that is complementary to the sgRNA, and the RuvC domain, which cuts the non-complementary strand. These two domains work together to create a double-strand break (DSB) at the intended target location. The PAM-interacting domain guarantees sequence specificity by recognizing the Protospacer Adjacent Motif (PAM), a brief DNA sequence located next to the target. This mechanism prevents Cas9 from targeting its own DNA while allowing for accurate differentiation of foreign sequences. (Doudna & Charpentier, 2014)

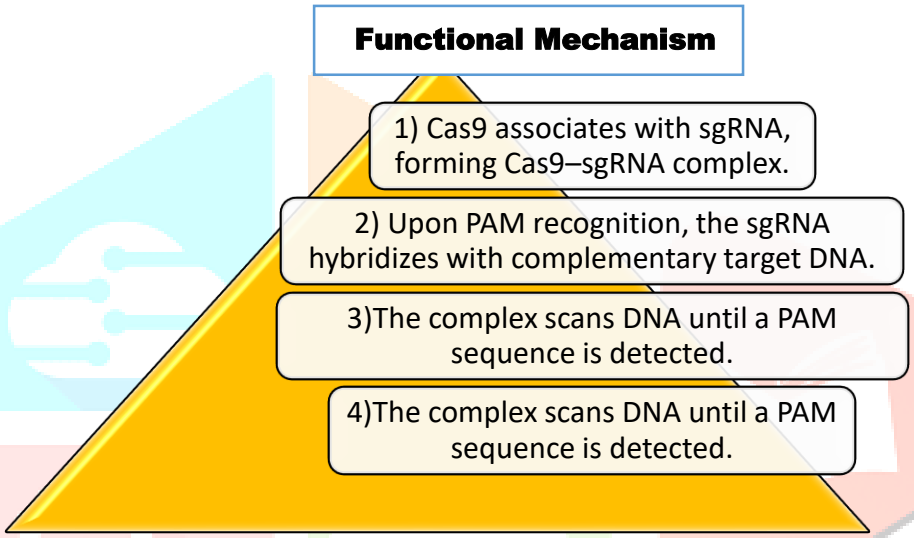

fig. 1 functional mechanisms crispr-cas9.

This structural and functional organisation underlies the versatility of Cas9 as a programmable tool for genome engineering, enabling applications in both research and therapeutic development.

A) Function of Guide RNA (gRNA) in Target Precision: The guide RNA (gRNA) serves as the essential element that directs Cas9 to the specific DNA sequence with great accuracy. In the engineered CRISPR-Cas9 system, the gRNA is a chimeric molecule that combines two native bacterial RNAs: the **CRISPR RNA (crRNA)**, which carries the sequence complementary to the target DNA, and the **trans-activating crRNA (tracrRNA)**, which facilitates binding to Cas9 (Jinek et al., 2012). This fusion simplifies the system into a single-guide RNA (sgRNA), making CRISPR-Cas9 adaptable for genome editing across diverse organisms. Immediately upstream of a Protospacer Adjacent Motif (PAM) sequence, the 20-nucleotide spacer region in the sgRNA base pairs with the complementary sequence of the target DNA. Thus, the presence of a PAM, usually "NGG" for Streptococcus pyogenes Cas9, and Watson–Crick base-pairing between the spacer and DNA dictate target specificity (Cong et al., 2013). The significance of sequence complementarity in specificity is highlighted by the fact that mismatches between the gRNA and the target DNA, especially in the seed region (the 8–12 nucleotides nearest to the PAM), can dramatically lower Cas9 binding and cleavage effectiveness (Hsu et al., 2013). Partial mismatches beyond the seed region, however, would still allow off-target activity, which would be problematic for therapeutic uses. To overcome these limitations, several approaches have been developed to enhance gRNA specificity, including the design of truncated sgRNAs, chemically modified gRNAs, and computational algorithms for predicting off-target sites (Fu et al., 2014; Doench et al., 2016). Together, these advances emphasise that gRNA plays a central role in determining both the **efficacy and fidelity** of CRISPR-Cas9-mediated genome editing. (Chapman et al., 2012; Sander & Joung, 2014).

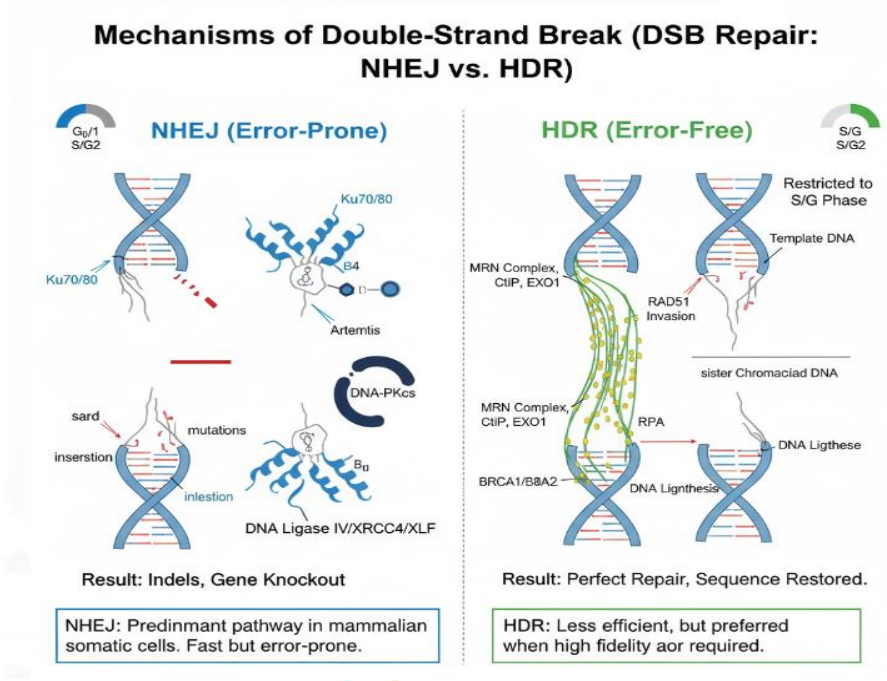

fig. 2 mechanisms of double-strand break.

A) Advances in Engineered Cas9 Variants:

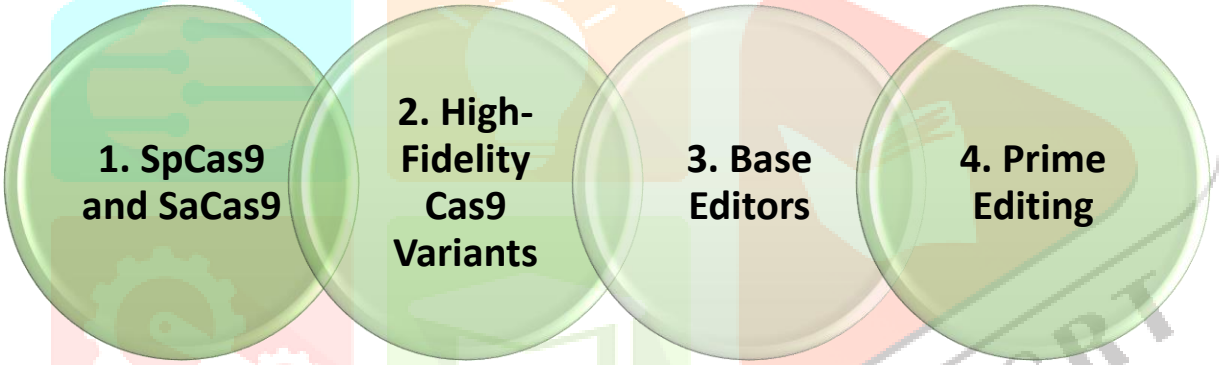

fig.3 advances in engineered cas9 variants.

1. SpCas9 and SaCas9

- **SpCas9**: The NGG PAM sequence is recognised by the most popular CRISPR nuclease, which is developed from Streptococcus pyogenes. Editing efficiency has increased due to the optimisation of guide RNA scaffolds (Jinek et al., 2012; Kleinstiver et al., 2015).

• **SaCas9**: Because of its compact size, this smaller Cas9 variant from Staphylococcus aureus with a NNGRRT PAM is appropriate for viral delivery (Ran et al., 2015).

2. Variants of High-Fidelity Cas9

• **eSpCas9**: Reduces off-target cleavage by introducing mutations in the HNH and RuvC domains (Slaymaker et al., 2016).

• **HypoCas9**: To further reduce off-target effects, it introduces further mutations that improve specificity (Chen et al., 2017).

• **SpCas9-HF1**: Modifies the RuvC domain to maintain high on-target efficiency while significantly lowering off-target activity (Kleinstiver et al., 2016).

3. Base Editors: Base editors are fusion proteins that enable accurate single-nucleotide conversions without double-strand breaks by combining cytidine or adenosine deaminases with a catalytically impaired Cas9 (dCas9 or nCas9). They have been used in therapeutic genome editing and targeted mutagenesis (Komor et al., 2016; Gaudelli et al., 2017).

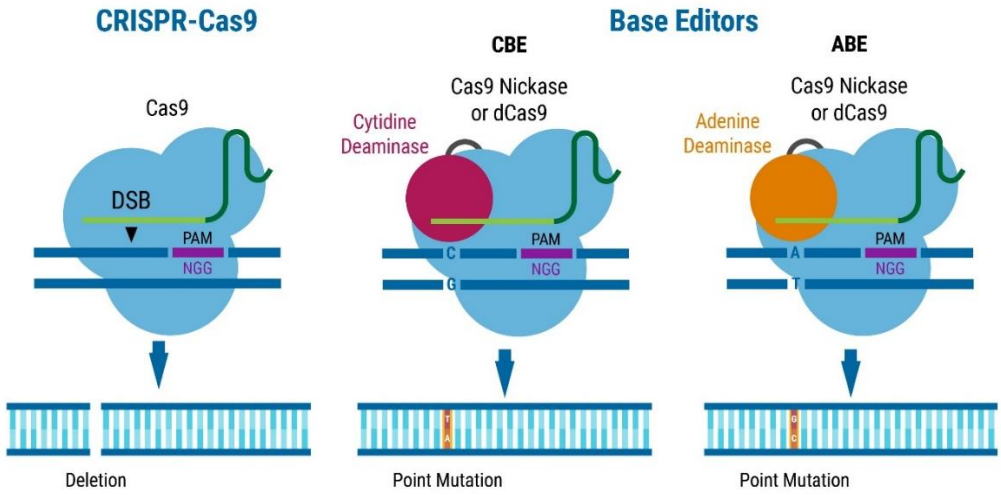

fig.4 base editors.

4. Prime Editing: Prime editing installs precise insertions, deletions, or substitutions without double-strand breaks by fusing a Cas9 nickase with a reverse transcriptase and a prime editing guide RNA (pegRNA). Recent advancements enhance the therapeutic applicability and editing efficiency (Anzalone et al., 2019; Lee et al., 2025).

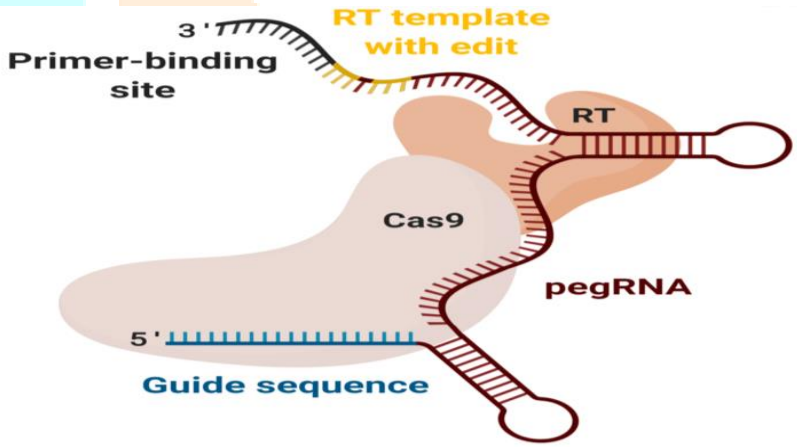

fig.5 prime editing.

3. Preclinical Applications of CRISPR-Cas9.

A) In Vitro Studies: Human Cell Lines and CRISPR-Cas9. Applying CRISPR-Cas9 to human cell lines has proven essential for comprehending disease processes and creating possible treatment plans. To investigate carcinogenesis, medication resistance, and therapeutic response, oncology researchers can use CRISPR-Cas9 to target the deletion or activation of oncogenes and tumour suppressor genes (Morgens et al., 2016). Novel vulnerabilities that can be used for anticancer therapy have been found by genome-wide CRISPR screenings in cancer cell lines (Aguirre et al., 2016). CRISPR-Cas9 has been utilised to fix disease-causing mutations in genetic diseases in vitro. For instance, the potential of CRISPR-Cas9 for individualised gene therapy was demonstrated by the successful repair of the ΔF508 mutation in the CFTR gene in airway epithelial cells obtained from patients with cystic fibrosis (Schwank et al., 2013).

Likewise, Duchenne Muscular Dystrophy treatment approaches have been uncovered through modifying DMD mutations in muscle cell lines (Ousterout et al., 2015). CRISPR-Cas9 has been used in infectious disease studies to interfere with host components that are essential for infection as well as integrated virus genomes. For example, proof-of-concept for CRISPR-based antiviral methods was shown by specifically disrupting latent HIV-1 proviral DNA in human T cells (Ebina et al., 2013). Additionally, there is promise for the development of curative medicines by CRISPR-mediated targeting of hepatitis B virus (HBV) cccDNA in hepatocyte cell lines (Seeger & Sohn, 2014). Induced pluripotent stem cells (iPSCs) derived from patients as preclinical models. iPSCs offer a potent in vitro platform for developing CRISPR-based therapies and simulating human illnesses. Reprogramming patient-derived iPSCs into disease-relevant cell types while maintaining the patient's

Patient-derived iPSCs are a helpful tool for researching genotype–phenotype correlations because they can be reprogrammed into disease-relevant cell types while maintaining the patient's genetic background (Soldner & Jaenisch, 2012). Mutations in iPSCs taken from patients with monogenic disorders such sickle cell disease, β-thalassemia, and Huntington's disease have been fixed using CRISPR-Cas9. Therapeutic potential can be demonstrated by the differentiation of corrected iPSCs into functional cell types (Xie et al., 2014; Li et al., 2015). In addition to gene correction, CRISPR-edited iPSCs are utilised to create isogenic controls, which improves mechanistic knowledge by enabling researchers to compare diseased and corrected cells under the same genetic background (Yusa, 2013). Thus, CRISPR-Cas9 applications in human cell lines and iPSC-derived models not only provide proof-of-concept for therapeutic approaches but also serve as preclinical platforms for safety, efficacy, and functional validation before moving into in vivo studies and clinical trials.

B) In Vivo Studies: Use in Animal Models-

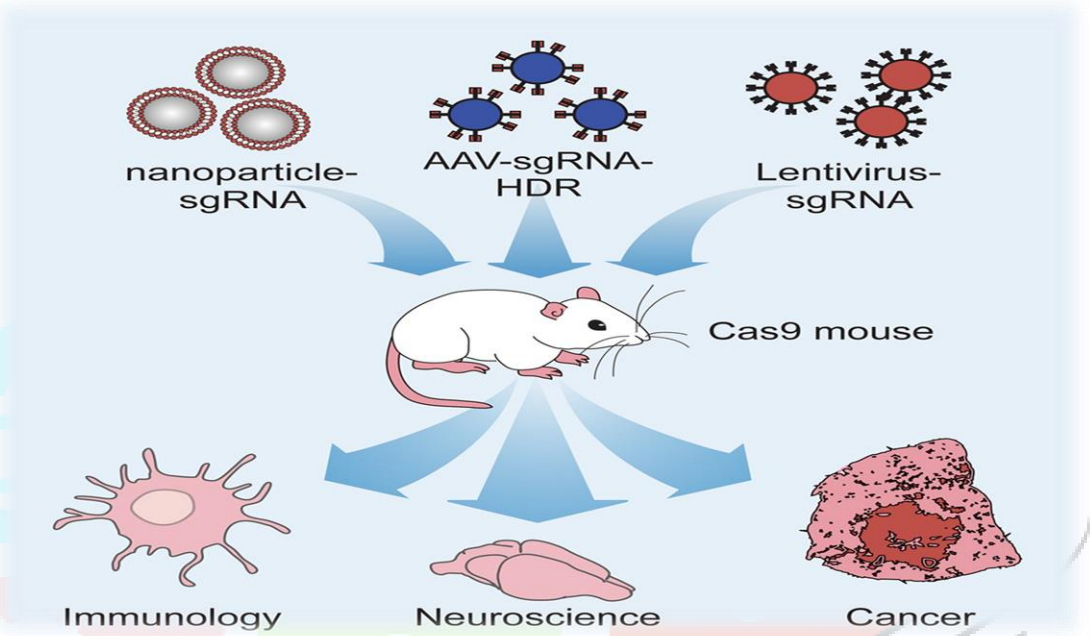

fig.6 animal models- in vivo studies.

Animal models have been indispensable for demonstrating the therapeutic potential of CRISPR-Cas9. In mice, systemic or tissue-specific delivery of Cas9 components has been used to edit genes involved in muscular dystrophy, liver diseases, and blood disorders (Long et al., 2016). Zebrafish provide a rapid and cost-effective model for functional studies, as CRISPR enables efficient generation of knockouts to investigate cardiovascular, developmental, and neurological phenotypes (Hwang et al., 2013). In non-human primates, CRISPR has been applied to generate models for metabolic and neurological disorders, providing translational relevance due to their closer similarity to humans (Niu et al., 2014).

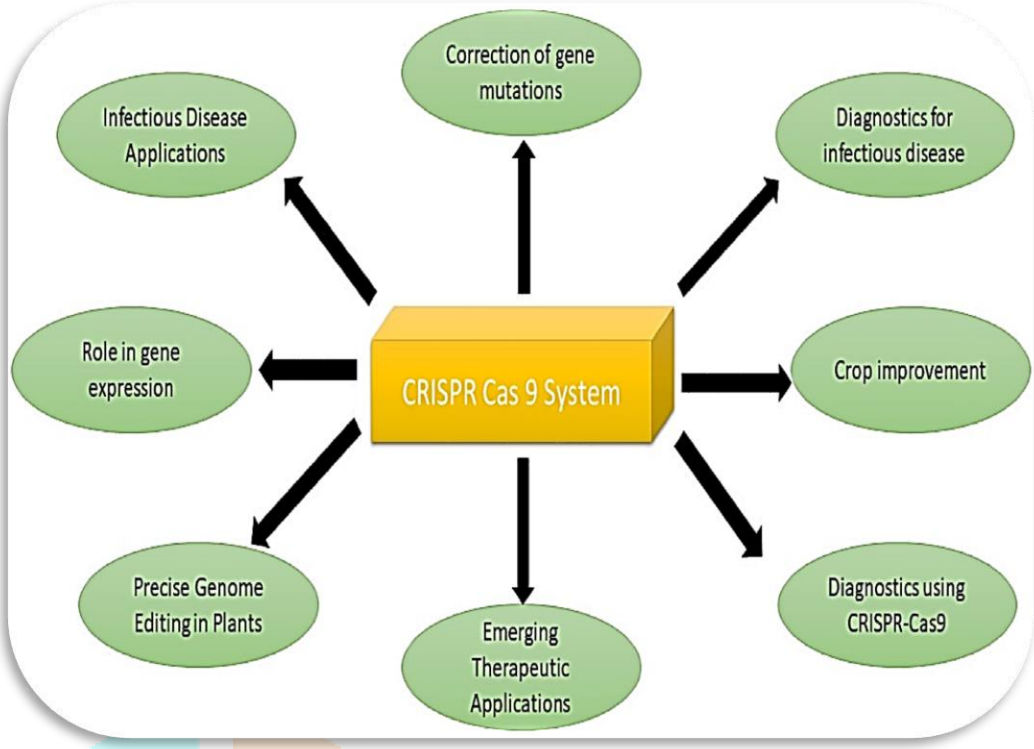

fig.7 applications of the crispr-cas9.

❖ **Proof-of-Concept in Monogenic Diseases**: In vivo, CRISPR-Cas9 has demonstrated potential in treating monogenic disorders. CRISPR-mediated exon skipping improved muscle function and restored dystrophin expression in a mouse model of Duchenne Muscular Dystrophy (DMD) (Nelson et al., 2016). Animal models of cystic fibrosis have shown functional recovery following CRISPR repair of the CFTR mutation in the airway epithelium (Schwank et al., 2013). The viability of genome editing for hereditary illnesses is demonstrated by these investigations.
ϖ Modelling Complex Diseases: Multifactorial diseases have also been modelled using CRISPR. New tumour suppressors and oncogenic drivers have been discovered in oncology by in vivo CRISPR screening in mouse models (Chen et al., 2015). Similarly, by introducing disease-related mutations into mice, CRISPR has been used to develop models of neurological diseases like Alzheimer's and Parkinson's disease.
❖ zebrafish, facilitating mechanistic studies and therapeutic testing (Yang et al., 2017).

C) Drug Discovery and Functional Genomics: CRISPR-Based Disease Modelling for Target Validation- CRISPR enables precise introduction of disease-associated mutations into preclinical models, helping validate targets in drug discovery pipelines. By creating isogenic cell lines or animal models with specific mutations, researchers can determine the causal role of genes in disease and evaluate their potential as therapeutic targets (Platt et al., 2014).

High-throughput CRISPR screens in preclinical research: It has transformed functional genomics by systematically identifying essential genes and druggable pathways. For example, CRISPR-based screens have revealed genes necessary for tumour cell survival, resistance mechanisms to targeted therapies, and host factors crucial for viral infections (Shalem et al., 2014; Wang et al., 2014). These high-throughput methods are now integrated into preclinical pipelines to guide rational drug development and precision medicine approaches.

4. Translational Advances: From Preclinical to Clinical Studies: A) Delivery Strategies: One of the biggest obstacles to therapeutic translation is still the effective and secure transport of CRISPR-Cas9 components to target cells and tissues. There are three main types of delivery strategies: physical techniques, non-viral carriers, and viral vectors. Regarding tropism, cargo capacity, immunogenicity, expression persistence, and manufacturability, each strategy has unique benefits and drawbacks.

Viral vectors (AAV, lentivirus)

Adeno-associated virus (AAV) vectors have become a dominant platform for in vivo gene delivery because of their favourable safety profile, broad tissue tropism (by serotype selection), and clinical track record in gene therapy. AAV efficiently transduces post-mitotic tissues (e.g., liver, muscle, retina) and has been used to deliver CRISPR components either by packaging Cas9 and guide RNAs (single or dual AAV systems) or by delivering donor templates for HDR. Limitations include small packaging capacity (~4.7 kb for AAV), which complicates delivery of larger Cas9 orthologs and regulatory elements, potential for long-term expression that can increase off-target risk, and preexisting anti-AAV immunity in patients. Lentiviral vectors (LV) provide larger cargo capacity and stable genomic integration, which is useful for ex vivo editing (e.g., edited hematopoietic cells) but carries insertional-mutagenesis risks that limit their use for in vivo somatic editing. Reviews: Wang et al., 2019; Ginn et al., 2018.

- **Non-viral delivery (lipid nanoparticles, polymer-based systems, exosomes)**

Non- viral systems are attractive for transient delivery of CRISPR components (mRNA, ribonucleoprotein complexes — RNPs), reducing long-term nuclease exposure and immune activation. Lipid nanoparticles (LNPs) have advanced rapidly — they enable systemic delivery of mRNA and RNP cargo and were successfully used in recent in vivo CRISPR therapeutic efforts (e.g., LNP-based delivery to the liver). Polymer-based nanoparticles, cell-penetrating peptides, and engineered extracellular vesicles/exosomes are also under development to improve tissue targeting and endosomal escape. Non-viral methods generally scale well for manufacturing and have lower insertional risk, but achieving efficient delivery to many tissues (e.g., brain, muscle) remains difficult. Reviews: Lino et al., 2018; Sago et al., 2018.

- **Physical methods (electroporation, microinjection, hydrodynamic delivery)**

Physical approaches remain valuable, especially for ex vivo editing or localised in vivo applications. Electroporation (or nucleofection) is widely used to deliver Cas9 RNPs or nucleic acids into cultured cells and primary cells, including T cells and hematopoietic stem/progenitor cells, enabling efficient editing for adoptive cell therapies. Microinjection is used for embryo editing and the generation of animal models. Hydrodynamic tail-vein injection (a high-volume, transient method) is used experimentally to deliver nucleic acids to the liver in rodents. Physical methods typically give high editing efficiency but are limited by invasiveness and poor applicability to many clinical contexts. Key points and tradeoffs are summarised in Table X (Delivery modality vs cargo, persistence, main uses, and limitations).

B) Safety and Efficacy Considerations: Off-target effects and genome integrity

Off-target DNA cleavage by Cas9 can introduce unintended mutations, chromosomal rearrangements, and large deletions, with potential genotoxic consequences. Off-target risk depends on guide RNA sequence, concentration and duration of Cas9 exposure, chromatin state, and cell type. Accurate assessment requires orthogonal detection methods (GUIDE-seq, Digenome-seq, SITE-seq, CIRCLE-seq, and deep targeted sequencing). Minimising off-targets is essential for clinical translation. (Hsu et al., 2013; Tsai & Joung, 2016).

C) Immune responses against Cas9 and delivery vehicles

Preexisting immunity to bacterial Cas9 proteins (e.g., from *S. pyogenes* or *S. aureus*) and immune responses against viral vectors (notably AAV) or LNP components may diminish efficacy and raise safety concerns. Adaptive immune responses can clear edited cells or trigger inflammation. Strategies to mitigate immune reactions include using transient delivery (RNPs, mRNA), evading humoral immunity via serotype selection or immunosuppression, engineering humanised or less-immunogenic nucleases, and choosing delivery routes and doses that minimise systemic exposure.

D)Strategies to minimise risks:

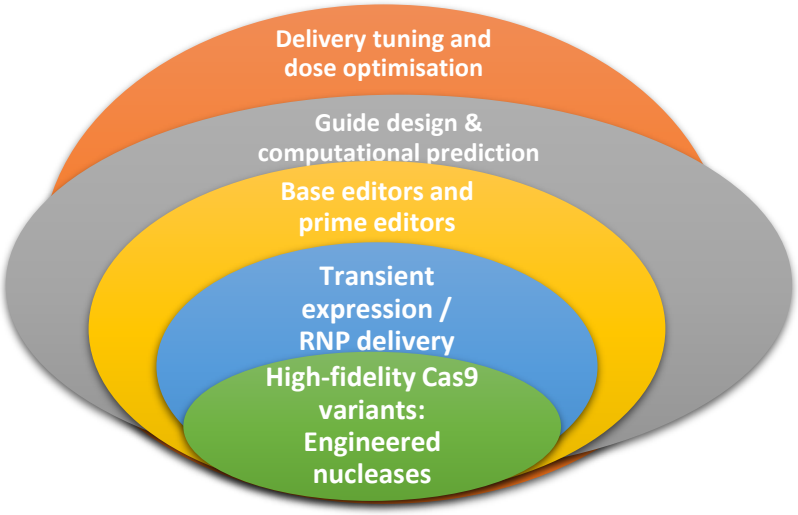

Fig.8 Strategies to minimise risks.

3) Regulatory and Ethical Considerations

➢ **Germline vs. somatic editing**

Regulatory and ethical frameworks draw a clear distinction between somatic cell editing (therapeutic changes confined to treated individuals) and germline editing (heritable changes that affect future generations). Somatic editing has advanced into clinical trials under existing regulatory pathways for gene therapies, whereas germline editing remains broadly discouraged or prohibited by many national bodies and international organisations due to profound ethical, safety, and societal implications.

➢ **International guidelines and ethical frameworks**

Multiple bodies have issued guidance on human genome editing. Key elements include phased translational oversight, rigorous preclinical evidence, long-term monitoring plans, transparent reporting and trial registries, and broad stakeholder engagement. International efforts — including reports and recommendations from national academies, the Nuffield Council on Bioethics, and the World Health Organisation (WHO) expert advisory groups —call for coordinated governance, responsible research practices, and limits on germline modification until safety, ethical, and societal issues are resolved**.**

➢ **Public perception and debates**

Public acceptance of genome editing varies by application (e.g., somatic therapy for severe disease vs. enhancement), cultural context, and trust in institutions. Concerns include safety, equity and access (who benefits), potential for misuse, and impacts on vulnerable populations. Transparent public dialogue, community engagement, and equitable policy frameworks are essential for aligning scientific advances with societal values.

5. Clinical Applications of CRISPR-Cas9:

table 1 clinical applications of crispr-cas9

Blood Disorders	Cancer Immunotherapy	Infectious Diseases	Emerging Clinical Applications
One of the most advanced clinical applications of CRISPR-Cas9 is in sickle cell disease (SCD) and β-thalassemia. CRISPR Therapeutics and Vertex developed exagamglogene autotemcel (exa-cel, CASGEVY™), an autologous hematopoietic stem cell therapy that disrupts the BCL11A enhancer to reactivate fetal haemoglobin. Clinical trial data demonstrated elimination of vaso-occlusive crises in nearly all treated SCD patients and durable transfusion independence in β-thalassemia (Frangoul et al., 2021; Vertex Press Release, 2023). In December 2023, CASGEVY became the first FDA-approved CRISPR-based therapy for SCD.	CRISPR is used to generate **CAR-T cells** with enhanced safety and function by knocking out endogenous TCRs and immune checkpoint genes. Clinical trials have tested multiplex-edited T cells in hematologic cancers and solid tumours (Stadtmauer et al., 2020). CRISPR-mediated disruption of **PD-1** has shown potential to improve antitumor responses, offering a genomic alternative to antibody-based checkpoint inhibitors.	CRISPR systems are being explored against persistent viral infections. In vitro, Cas9 has been used to excise integrated **HIV-1 provirus** and disrupt essential **HBV cccDNA**, while Cas13-based systems are under investigation for RNA viruses, including **SARS-CoV-2** (Xu et al., 2019; Abbott et al., 2020). While clinical translation is at an early stage, these approaches highlight CRISPR's potential for antiviral therapy.	CRISPR-based therapies are being investigated for **neurological disorders** (e.g., *HTT* gene correction in Huntington's, *SOD1* in ALS) and **cardiovascular/metaboli c diseases** (e.g., *PCSK9* disruption to reduce LDL cholesterol). While these remain in preclinical or early trial stages, they illustrate CRISPR's potential to extend beyond monogenic conditions into complex, multifactorial diseases (Musunuru et al., 2021).

6. Challenges and Limitations

Despite the rapid advances and clinical successes of CRISPR-Cas9, significant challenges remain before the technology can be widely adopted as a safe and reliable therapeutic platform. These limitations fall into technical, biological, translational, and ethical/societal domains.

- **Technical Barriers**

- **Editing efficiency:** Variable efficiency across cell types and genomic loci remains a barrier to reproducibility and therapeutic effectiveness. Factors such as chromatin accessibility, guide RNA design, and repair pathway activity influence editing success (Hsu et al., 2013).

- **Specificity:** Off-target effects can lead to undesired mutations, chromosomal rearrangements, or genotoxicity. Despite improvements with high-fidelity Cas9 variants, base editors, and prime editors, achieving absolute specificity remains challenging (Kleinstiver et al., 2016).

- **Mosaicism:** In vivo editing, particularly in early developmental or germline contexts, may result in mosaicism, where not all cells carry the intended edit, complicating therapeutic outcomes (Mehravar et al., 2019).

- **Biological Barriers**

- **Delivery:** Efficient, tissue-specific delivery of CRISPR components remains one of the greatest bottlenecks. While AAV, LNPs, and electroporation are commonly used, achieving safe and widespread distribution to organs like the brain, heart, or lungs is still limited (Lino et al., 2018).

- **Immune responses:** Pre-existing immunity to bacterial Cas9 proteins (*S. pyogenes* and *S. aureus*) as well as host responses to viral vectors or nanoparticles pose risks for both efficacy and safety (Charlesworth et al., 2019).

- **Long-term safety:** Persistent Cas9 expression, insertional mutagenesis (viral delivery), or unintended genomic alterations raise concerns about tumorigenicity and genomic instability. Longitudinal studies are needed to establish safety profiles.

- **Translational Barriers**

- **Scaling from preclinical to human studies:** Many therapeutic concepts that show promise in animal models face difficulties in translation due to interspecies differences, variability in repair pathway activity, and delivery barriers in humans (Yin et al., 2017).

- **Manufacturing and cost:** Producing clinical-grade CRISPR components (Cas9 proteins, sgRNAs, delivery systems) at scale and ensuring quality control remains a challenge, particularly for ex vivo autologous therapies like edited HSCs or T cells.

- **Regulatory oversight:** Regulatory frameworks must adapt to the unique features of genome editing, including long-term monitoring of patients, germline risks, and off-target surveillance.

- **Ethical and Societal Challenges**

- **Equity and accessibility:** High costs of CRISPR-based therapies raise concerns about global accessibility, particularly in low- and middle-income countries, where the burden of genetic diseases may be high. Without equitable distribution, these innovations risk widening health disparities (Baylis, 2019).

- **Germline editing controversies:** Heritable genome editing remains one of the most contentious issues in biomedical ethics. The 2018 birth of CRISPR-edited babies in China sparked global debates on safety, governance, and societal implications (Cyranoski, 2019). While international guidelines recommend moratoria on germline editing, societal pressure for enhancement applications may increase in the future.

- **Public perception:** Societal trust, informed consent, and transparent communication are critical for public acceptance of genome editing. Misuse or premature application of CRISPR could undermine its legitimacy as a therapeutic platform.

7. Future Perspectives

- The future of CRISPR-based medicine lies in improving precision, expanding editing modalities beyond DNA cleavage, and integrating with complementary technologies to advance personalised and precision healthcare. Several next-generation strategies are shaping the translational landscape.

A) Next-Generation CRISPR Systems (Cas12, Cas13, Cas14)

- Beyond Cas9, newly characterised CRISPR effectors provide alternative editing capabilities:

- **Cas12a (Cpf1)**: Recognises T-rich PAMs, generates staggered DNA cuts, and requires only a single crRNA, making it valuable for multiplex editing (Zetsche et al., 2015).

- **Cas13**: Targets RNA rather than DNA, enabling transcriptome editing without permanent genomic alterations. Cas13-based systems are being explored for RNA virus detection and therapy, including SARS-CoV-2 diagnostics (Abudayyeh et al., 2017).

- **Cas14**: Ultralight nucleases (<1,000 amino acids) that can target single-stranded DNA, offering potential for compact delivery systems (Harrington et al., 2018).

B) Base and Prime Editing in Therapeutic Development

- Double-strand breaks, which are necessary for traditional Cas9, can result in indels and genomic instability. Point mutations that cause a lot of genetic illnesses can be corrected thanks to base editors (cytidine and adenine base editors), which allow for the direct, irreversible conversion of single nucleotides without DSBs (Komor et al., 2016; Gaudelli et al., 2017). By facilitating targeted insertions, deletions, and base modifications mediated by a reverse transcriptase-Cas9 fusion and a prime editing gRNA, prime editing further broadens capabilities (Anzalone et al., 2019). With improved safety profiles, both methods have the potential to cure monogenic illnesses that were previously untreatable. B) CRISPR-Based Transcriptome and Epigenome Editing programmed control of gene expression without changing the underlying DNA sequence is made possible by catalytically inactive Cas9 (dCas9) coupled to transcriptional activators, repressors, or epigenetic modifiers (Gilbert et al., 2013).
- Similarly, Cas13-based tools can modulate RNA splicing and stability. These modalities may provide safer alternatives for therapeutic intervention by offering reversible and tunable regulation of disease-relevant pathways.

B) Potential for Personalised and Precision Medicine

The development of isogenic disease models and the correction of patient-specific mutations are enabled by CRISPR platforms, opening the door for personalized treatments. When combined with organoid systems and induced pluripotent stem cells, CRISPR can be used to predict therapy outcomes and develop patient-specific therapies. The shift from generalized treatments to individualized genomic medicine may result from integrating these approaches into clinical practice (Schmidt & de Vries, 2020).

C) Integration with AI, Nanotechnology, and Synthetic Biology

- The next frontier of CRISPR innovation lies in combining it with other transformative technologies:
- **Artificial Intelligence (AI):** Machine learning is being used to predict gRNA activity, minimize off-target effects, and accelerate therapeutic design pipelines (Chuai et al., 2018).
- **Nanotechnology:** Advanced nanocarriers, including lipid-based, polymeric, and inorganic nanoparticles, offer precise delivery to otherwise inaccessible tissues (Lee et al., 2017).
- **Synthetic Biology:** CRISPR components can be engineered into synthetic circuits for controlled gene expression, biosensing, and therapeutic responses (Nielsen & Voigt, 2014).

8. Conclusion

From a bacterial defense mechanism, the CRISPR-Cas9 system has swiftly developed into one of the most ground-breaking innovations in contemporary biomedicine. Its capacity to correct monogenic errors, mimic complicated diseases, and identify novel therapeutic targets has been validated by preclinical research conducted in cell lines, iPSCs, and animal models. Early clinical successes have resulted from these advancements, particularly in blood diseases, where exagamglogene autotemcel (CASGEVYTM), the first CRISPR-based treatment, was approved by the FDA, marking a significant milestone. The platform's adaptability is further demonstrated by ongoing trials in infectious diseases, cancer, and uncommon genetic diseases (EDIT-101, NTLA-2001).

However, there are several restrictions using CRISPR-Cas9. Before widespread adoption, technical issues such biological and immunological barriers, off-target editing, mosaicism, and delivery efficiency must be resolved.

The advent of next-generation editing techniques, like as epigenome modulators, RNA-targeting CRISPR systems, and base and prime editors, presents encouraging ways to improve accuracy and safety. Combining CRISPR with artificial intelligence, nanotechnology, and synthetic biology could increase its use in precision and customized medicine.

In the end, CRISPR-Cas9 is a groundbreaking platform that has the potential to transform the way complicated and genetic disorders are treated, in addition to being a therapeutic tool. However, its use necessitates a balanced strategy: taking use of its potential while remaining vigilant about long-term safety, access fairness, and ethical bounds, especially with regard to germline modification. To guarantee that this potent technology is developed ethically, safely, and for the benefit of all, scientists, physicians, regulators, ethicists, and society as a whole will need to work closely together as clinical translation develops.

References -

1. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered

2. zinc finger nucleases. *Nat Rev Genet*. 2010;11(9):636–46. https://doi.org/10.1038/nrg2842

3. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol*. 2013;14(1):49–55. https://doi.org/10.1038/nrm3496

4. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816–21. https://doi.org/10.1126/science.1225829

5. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;339(6121):819–23. https://doi.org/10.1126/science.1231143

6. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007;315(5819):1709–12. https://doi.org/10.1126/science.1138140

7. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. *Science*. 2013;339(6121):823–6. https://doi.org/10.1126/science.1232033

8. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016;533(7603):420–4. https://doi.org/10.1038/nature17946

9. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 2019;576(7785):149–57. https://doi.org/10.1038/s41586-019-1711-4

10. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*. 2014;343(6166):84–7. https://doi.org/10.1126/science.1247005

11. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. *Science*. 2014;343(6166):80–4. https://doi.org/10.1126/science.1246981

12. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096. https://doi.org/10.1126/science.1258096

13. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816–21. https://doi.org/10.1126/science.1225829

14. Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell*. 2014;156(5):935–49. https://doi.org/10.1016/j.cell.2014.02.001

15. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096. https://doi.org/10.1126/science.1258096

16. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816–21. https://doi.org/10.1126/science.1225829

17. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;339(6121):819–23. https://doi.org/10.1126/science.1231143

18. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol*. 2013;31(9):827–32. https://doi.org/10.1038/nbt.2647

19. Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, Sander JD. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. 2013;31(9):822–6. https://doi.org/10.1038/nbt.2623

20. Doench JG, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, et al. Optimised sgRNA design to maximise activity and minimise off-target effects of CRISPR-Cas9. *Nat Biotechnol*. 2016;34(2):184–91. https://doi.org/10.1038/nbt.3437

21. Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell*. 2012;47(4):497–510.
22. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096), 816–821.
23. Kleinstiver, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z., & Joung, J.K. (2016). High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, 529, 490–495.
24. Slaymaker, I.M., Gao, L., Zetsche, B., Scott, D.A., Yan, W.X., & Zhang, F. (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science*, 351(6268), 84–88.
25. Chen, J.S., Dagdas, Y.S., Kleinstiver, B.P., Welch, M.M., Sousa, A.A., Harrington, L.B., … & Doudna, J.A. (2017). Enhanced proofreading governs the accuracy of CRISPR–Cas9 targeting. *Nature*, 550, 407–410.
26. Ran, F.A., Cong, L., Yan, W.X., Scott, D.A., Gootenberg, J.S., Kriz, A.J., … & Zhang, F. (2015). In vivo genome editing using Staphylococcus aureus Cas9. *Nature*, 520, 186–191.
27. Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A., & Liu, D.R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 533, 420–424.
28. Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., & Liu, D.R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, 551, 464–471.
29. Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., … & Liu, D.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576, 149–157.
30. Morgens DW, Deans RM, Li A, Bassik MC. Systematic comparison of CRISPR/Cas9 and RNAi screens for essential genes. *Nat Biotechnol*. 2016;34(6):634–6. https://doi.org/10.1038/nbt.3567
31. Aguirre AJ, Meyers RM, Weir BA, Vazquez F, Zhang CZ, Ben-David U, et al. Genomic copy number dictates a gene-independent cell response to CRISPR-Cas9 targeting. *Cancer Discov*. 2016;6(8):914–29. https://doi.org/10.1158/2159-8290.CD-16-0154
32. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell*. 2013;13(6):653–8. https://doi.org/10.1016/j.stem.2013.11.002
33. Ousterout DG, Kabadi AM, Thakore PI, Majoros WH, Reddy TE, Gersbach CA. Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations. *Nat Commun*. 2015;6:6244. https://doi.org/10.1038/ncomms7244
34. Ebina H, Misawa N, Kanemura Y, Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. *Sci Rep*. 2013;3:2510. https://doi.org/10.1038/srep02510
35. Seeger C, Sohn JA. Targeting hepatitis B virus with CRISPR/Cas9. *Mol Ther Nucleic Acids*. 2014;3:e216. https://doi.org/10.1038/mtna.2014.68
36. Soldner F, Jaenisch R. iPSC disease modeling. *Science*. 2012;338(6111):1155–6. https://doi.org/10.1126/science.1227682
37. Xie F, Ye L, Chang JC, Beyer AI, Wang J, Muench MO, Kan YW. Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. *Genome Res*. 2014;24(9):1526–33. https://doi.org/10.1101/gr.173427.114
38. Li HL, Fujimoto N, Sasakawa N, Shirai S, Ohkame T, Sakuma T, et al. Precise correction of the Huntington's disease mutation in patient-derived iPSCs by Cas9 and CRISPR. *Cell Stem Cell*. 2015;17(3):303–13. https://doi.org/10.1016/j.stem.2015.07.001
39. Yusa K. Seamless genome editing in human pluripotent stem cells using CRISPR/Cas9 and piggyBac. *Nat Protoc*. 2013;8(12):2061–78. https://doi.org/10.1038/nprot.2013.126

39. Long C, Amoasii L, Mireault AA, McAnally JR, Li H, Sanchez-Ortiz E, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science*. 2016;351(6271):400–3. https://doi.org/10.1126/science.aad5725

40. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*. 2013;31(3):227–9. https://doi.org/10.1038/nbt.2501

41. Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell*. 2014;156(4):836–43. https://doi.org/10.1016/j.cell.2014.01.027

42. Nelson CE, Hakim CH, Ousterout DG, Thakore PI, Moreb EA, Castellanos Rivera RM, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science*. 2016;351(6271):403–7. https://doi.org/10.1126/science.aad5143

43.Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096. https://doi.org/10.1126/science.1258096

44. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol*. 2014;32(4):347–55. https://doi.org/10.1038/nbt.2842

45. Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR–Cas9 gene editing for sickle cell disease and β-thalassemia. *N Engl J Med*. 2021;384(3):252–60. https://doi.org/10.1056/NEJMoa2031054

46. Vertex Pharmaceuticals. Vertex and CRISPR Therapeutics announce FDA approval of CASGEVY™ (exagamglogene autotemcel). 2023. https://investors.vrtx.com/news-releases/news-release-details/vertex-and-crispr-therapeutics-announce-us-fda-approval

47.Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. *Science*. 2020;367(6481):eaba7365. https://doi.org/10.1126/science.aba7365

48. Xu CL, Ruan MZC, Mahajan VB, Tsang SH. Viral delivery systems for CRISPR. *Viruses*. 2019;11(1):28. https://doi.org/10.3390/v11010028

49. Abbott TR, Dhamdhere G, Liu Y, Lin X, Goudy L, Zeng L, et al. Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza. *Cell*. 2020;181(4):865–76.e12. https://doi.org/10.1016/j.cell.2020.04.020

50. Maeder ML, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nat Med*. 2019;25(2):229–33. https://doi.org/10.1038/s41591-018-0295-0

51. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol*. 2013;31(9):827–32. https://doi.org/10.1038/nbt.2647

52. Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, et al. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*. 2016;529(7587):490–5. https://doi.org/10.1038/nature16526

53. Mehravar M, Shirazi A, Nazari M, Banan M. Mosaicism in CRISPR/Cas9-mediated genome editing. *Dev Biol*. 2019;445(2):156–62. https://doi.org/10.1016/j.ydbio.2018.10.008

54. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv*. 2018;25(1):1234–57. https://doi.org/10.1080/10717544.2018.1474964

55. Charlesworth CT, Deshpande PS, Dever DP, Camarena J, Lemgart VT, Cromer MK, et al. Identification of pre-existing adaptive immunity to Cas9 proteins in humans. *Nat Med*. 2019;25(2):249–54. https://doi.org/10.1038/s41591-018-0326-x

56. Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. *Nat Rev Drug Discov*. 2017;16(6):387–99. https://doi.org/10.1038/nrd.2016.280

57. Baylis F. Human genome editing: our future belongs to all of us. *Trends Genet*. 2019;35(7):451–4. https://doi.org/10.1016/j.tig.2019.04.005

58. Cyranoski D. CRISPR-baby scientist fails to satisfy critics. *Nature*. 2019;566(7742):161–2. https://doi.org/10.1038/d41586-019-00673-1

59. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR–Cas system. *Cell*. 2015;163(3):759–71. https://doi.org/10.1016/j.cell.2015.09.038

60. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DBT, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*. 2017;353(6299):aaf5573. https://doi.org/10.1126/science.aaf5573

61. Harrington LB, Burstein D, Chen JS, Paez-Espino D, Ma E, Witte IP, et al. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. *Science*. 2018;362(6416):839–42. https://doi.org/10.1126/science.aav4294

62. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-strand DNA cleavage. *Nature*. 2016;533(7603):420–4. https://doi.org/10.1038/nature17946

63. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*. 2017;551(7681):464–71. https://doi.org/10.1038/nature24644

64. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 2019;576(7785):149–57. https://doi.org/10.1038/s41586-019-1711-4

65. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*. 2013;154(2):442–51. https://doi.org/10.1016/j.cell.2013.06.044

66. Schmidt F, de Vries H. From personalised medicine to precision medicine in oncology. *Chin Clin Oncol*. 2020;9(2):15. https://doi.org/10.21037/cco.2019.12.07

67. Chuai G, Ma H, Yan J, Chen M, Hong N, Xue D, et al. DeepCRISPR: optimised CRISPR guide RNA design by deep learning. *Genome Biol*. 2018;19:80. https://doi.org/10.1186/s13059-018-1459-4

68. Lee B, Lee K, Panda S, Gonzales-Rojas R, Chong A, Bugay V, et al. Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nat Biomed Eng*. 2017;1(11):889–901. https://doi.org/10.1038/s41551-017-0137-2

69. Nielsen AA, Voigt CA. Multi-input CRISPR/Cas genetic circuits that interface host regulatory networks. *Mol Syst Biol*. 2014;10:763.