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Review Article

CRISPR-Based Therapeutics: Clinical Applications, Challenges, and Future Perspectives: A Review

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ABSTRACT

CRISPER-Cas9 has emerged as a clinically approved therapeutic platform exemplified by the recent 2023 approval of Casgevy , for sickle cell disease and ß- thalassemia is a watershed achievement. This technology now encompasses ex vivo hematopoietic editing, on vivo systemic delivery, cancer immunotherapy, and there are new as well as potential applications in cardiovascular, neurological, and infectious diseases. The clinical translation of the science appears most far advanced in the area of haematology where hematopoietic stem cells can be isolated andedited ex vivo and assessed before reinfusion . Oncology examples have shown that allogeneic CAR-T cells using CRISPR editing can yield high response rates on hematologic malignancies, and achieved, by far, a durable complete remission for a solid tumor indication. Systemic in vivo editing has demonstrated proof of concept in the treatment of hereditary transthyretin amyloidosis, broadening the scope of the therapeutic model beyond ex vivo applications. Human trails of base editing are underway with VERBE-101 demonstrating sustained 50% LDL cholesterol reduction following a single dose the first clinical proof of principle for this modality .

Genome editing based on CRISPR has swiftly evolved from an adaptive immunity mechanism of microbes into a clinically proven therapy that enables permanent manipulation of disease causing sequences. The present review covers the clinical implementation, current translational challenges, and future directions of CRISPR therapies in human medicine. Addressing the challenges of conventional cleavage medicated by nucleases is dependent on the design of precision engineered genome editors. Additionally, reversible transcriptional regulation through temporary epigenome editing and RNA targeting via Cash 13 effectors are emerging fields in CRISPR technologies

Keywords: CRISPER-Cas9, Ex vivo editing ,In vivo Delivery, Base editing, Epigenome editing , Cas13 RNA targeting, Hematology, Oncology and Hereditary amyloidosis

INTRODUCTION:

Need for precise genome-editing tools:

Creating accurate genome editing tools is critical to increase the safety, efficiency and accuracy of genetic editing. Despite the power of CRISPR-Cas genome editing tools, advances to overcome the limitations such as off-target editing, unwanted modifications to DNA and decreasing efficiency need to be achieved to allow specific target sequences in DNA to be targeted. These tools may ultimately enable improved editing for genetic research, disease models, future gene therapies and for crop improvement.Genome editing, a technique that precisely and efficiently modify DNA sequences can be added to in the human genome to manipulate cell fates and can potentially cure genetic diseases. [4,5,6,7,8,9]

In 2012,Bacterial clustered regularly interspaced short palindromic repeats.(CRISPR)-CRISPR-associated protein 9 (Cas9) was designed for RNA Programmable DNA cutting.Since then, CRISPR-Cas systems have been employed to modify genome of Cultured primary cells animals and plants, resulting in major technological break throughs in the life sciences and

Being studied in several clinical trials. With the proliferation of the are aware of the impressive number of new CRISPR-Cas types, we focus on CRISPR-Cas9/Cas12 in this is for the most commonly used genome editors.CRISPR-Cas DNA editing creates double-strand breaks (DSBs) that are repaired predominantly by non-homologous end-joining pathway (NHEJ) and homology-directed repair (HDR). Whereas NHEJ small mutations at the target sites in the form of insertions or deletions (indels) or gene disruption, homology-directed repair (HDR) can create.Sequence to the exogenous DNA template at the DSB locus, although the efficiency can be low and it is turned off in quiescent cells. As opposed to, base editors and prime editors make specific single-nucleotide changes to the genome without requiring DSBs or donor DNA templates.

CRISPR-associated transposases, Tn6677 and CAST, allow for larger DNA cargos, but thus far have only demonstrated activity in bacterial cells. In a nutshell, this system involves various combinations of components to insert up to 10 kb of desired cargos at target loci, the transposase operon (TnsA, TnsB, TnsC, TniQ), donor transposase DNA substrate and CRISPR-based DNA binding and R-loop-generating proteins (cas6, cas7, and cas8-cas5. Fusion genes and individual CRISPR array for Tn6677 or the Cas12k and the Cas12k guide

RNA array for CAST. Engineered Cas9-"fused integrase/recombinase, which can in insert, delete, invert" or replace target DNA have been also been reported to alter genomic DNA in a mammalian cell, although at present it remains inefficient and highly constrained by target sequence requirements. Along with DNA editing, adenosine deaminases working on RNA (ADAR) fused to dCas13 have been used to accomplish accurate. Adenosine-to-inosine editing of target RNA20. Whereas genome editing creates fixed mutations in the DNA sequence, RNA editing is temporary and reversible.Targeted RNA editing can alternatively be achieved through recruitment.Endogenous ADARs using synthetic ADAR-recruiting RNAs, circumventing the requirement to deliver exogenous editing enzymes. ADARs proteins into edited cells. The potential of therapeutics as part of a complementary approach to genomes editing.In this review, we discuss the recent advances in the progress and use of CRISPR-based genome editing and RNA editing systems,including CRISPR-Cas9/Cas12/Cas13 nucleases, DNA base editors, prime editors, and RNA base editors, which are further discussed to refine their editing capabilities for future applications.Enabling for high-capacity editing efficiency and accuracy. We review summarizes different NGS-based techniques that have been developed to investigate unintended genetic adjustments and look into the safety issues during gene editing. [17,18,19,20]

Evolution of CRISPR from bacterial immunity to therapeutic platform:

CRISPR is part of the immune system of bacteria and archaea. It helps to defend these single-celled organisms from attacks by viruses (or bacteriophages) and other foreign genetic material.Researchers took this natural immune system and engineered it into a powerful genome-editing technology to cut DNA at precise locations. Now, people use CRISPR to learn more about how the world works, cure genetic diseases, and improve agriculture and biotechnology.Clustered Regularly Interspaced Short Palindromic Repeats

The CRISPR related to which bacteria was identified in E coli and was described as a 1,664-nucleotide sequence by Ishino in 1987.Six years later, Mojica and Van Soolingen found similar DNA fragments in Haloarcula and HaloferaxArchaea and M.tuberculosis separately.By 2000, Mojica's team had discovered CRISPR DNA sequences in 20 bacterial species. After the advent of DNA sequencing techniques, the wider presence of CRISPR was reported in 2005: most archaea (90%) and some bacteria (40%) possess CRISPR DNA. Francisco Mojica, a Spanish scientist, together with his colleagues, characterized the CRISPR sequence and introduced the term "clustered regularly interspaced short palindromic repeats" (CRISPR) to refer to it as a bacterial immune system (Jacinto et al., 2020).However, till 2007, the functions of the CRISPR still remained unknown in spite of its widely discovery in the first two decades. Prior to clearing the functions, two important studies accelerated the research: the neighboring genes to the CRISPR locus 2002, and the foreign viral DNA in the CRISPR spacers 2005 , which directly pushed forward the proposing of the hypothesis that the CRISPR system was an innate immune system that archaea and bacteria used to defend against invading viruses.Finally, in 2007, the hypothesis was confirmed by the experiments in Barrangou's lab, and the functions of the 'weird' CRISPR were finally unveiled.Due to the perfect ability of its target searching and cleavage to DNA sequence, this system was designed as a gene editing tool in 2012. When compared with the previous gene editing tools such as zinc finger nuclease (ZFN) and transcription activator-like effector nucleases (TALENs), this gene editing tool based on CRISPR performed far better. The most important thing is that this gene editing system is very easy for researchers to design, which makes it remarkably easier to be applied. Now, using this system (CRISPR/Cas9), the gene editing system becomes more and more common all over the world. The first clinical application of the system is carried out at the West China Hospital in China in 2016 by injecting the CRISPR/Cas9 edited cells to treat aggressive lung cancer. In recent years, many clinical trials are being processed, and some results have been published, for example, the treatment of acquired immunodeficiency syndrome (AIDS), sickle cell disease (SCD), β-thalassemia, and different kinds of cancers. For the development of CRISPR/Cas9 gene editing technique, Emmanuelle Charpentier and Jennifer Doudna got the Nobel Prize in 2020.This gene-editing tool, which has been described as a pair of genetic scissors, could perform precise modifications to the DNA. The inventors then identified that this natural biological process could be exploited to cut and remove defective genes and introduce working copies, heralding the future of medicine (Strzyz, 2020). They also identified a second, short RNA, trans activator CRISPR RNA (tracrRNA), which was essential to the CRISPR-Cas system, reconstructed all the necessary CRISPR-Cas molecules for precise cutting of the target, and proposed combining each CRISPR RNA (crRNA) with the tracrRNA into a single guide RNA (sgRNA) thus is the first proposal to use these tools for RNA-programmable genome editing based on RNA-guided DNA cutter cleavage system (Kim and Kim, 2014). Doudna also helped elucidate the protein structures underlying the Dnase activity, and RNA processing for the CRISPR-Cas system. In Nobel awarding the 2020 Nobel Prize in chemistry to them, and their pioneering efforts in discovering and developing CRISPR into a new tool for editing genomes, the two women became the first in history to share a Nobel prize (Uyhazi and Bennett, 2021). Since this discovery, large technological advances have allowed for use within several sectors of biotechnology and medicine including microbiology, and this review seeks to provide an overview of some recent developments of this innovative tool. [1,2,3,4,7]

Rationale, scope, and objectives of the review:

Introduction state the reason why you feel that need for this CRISPR review.Genome editing techniques: DNA in gene-editing techniques can be altered easily and efficiently using today's techniques. Gene editing involves the insertion, deletion or substitution of specific DNA sequences in the genome of an organism. CRISPR-Cas is one such CRISPR gene editing technology.Interest in CRISPR science for the treatment of disease has been

prevalent since the discovery of CRISPR as part of the adaptive immune system in bacteria and archaea, leading to its evolution as an emerging platform for genetic research and medicine. As such, summarising its history, mechanism, clinical applications and limitations is relevant. [7,8,9,10,20]

Evolution and advancement of CRISPR Technology:
Introduction The story of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) involves the history of its discovery and how the technology plays into bacteria and archea's immune system. Microorganisms generate the ability to recognize and defend against intruding genetic material through the use of CRISPR-associated (Cas) proteins that can target foreign DNA like a bacteriophage. This natural immune system is how we can utilize it as a tool to engineer any select gene in the DNA. [1,2,3,4]

The Molecular Mechanics of CRISPR-Cas Systems: [4,7,8,10]
This review describes the molecular mechanisms involved in the recognition and editing of the target DNA sequence by the CRISPR system. Guide RNA, Cas enzymes, the recognition of target sequences, and the break in DNA are explained. For the CRISPR-Cas9 method, the guide RNA molecule recognizes the complement DNA sequence and, with the help of the Cas9 protein, creates a break in the DNA. The DNA repair pathways of a cell can then be used to gene knockout or insertion.

Types and Classification of CRISPR Systems: [11,12,13,14,15,16]
The important types of CRISPR-Cas systems are introduced, their different properties are discussed. Mostly, CRISPR-Cas9 as a tool for genome editing and CRISPR-Cas12 with DNA editing function and molecular diagnostic applications are explained, then CRISPR-Cas13 which specifically targets RNA are explained. These systems which have different molecular targets, molecular properties and applications can be used to develop tools for different research and therapeutic approaches.

Advances in Precise Genome-Editing Tools:
Coverage extends to new genome-editing methods that are intended to offer increased accuracy, efficiency, or safety, such as base editing, which has the ability to make one-letter DNA edits without inducing a double stranded break; and prime editing, which introduces desired edits to DNA with a guide RNA and engineered reverse transcriptase. The review also looks at improvements in guide RNA design and editing specificity, and approaches to avoid unintended or unwanted editing. These improvements seek to overcome some of the limitations of conventional CRISPR-Cas9 editing. [17,18,19,20,43,44]

Using CRISPR Technology for Therapies: CRISPR technologies in medicine. It considers the potential of CRISPR to treat sickle cell disease and beta-thalassemia, and explored work into cancer, inherited diseases, and specific infectious diseases. It discusses off-the-shelf ex vivo editing (editing performed outside the body) and in vivo editing (treatments delivered directly into the body) and also explains which of these clinical procedures are already in use and which still require more research.

CRISPR in Molecular Diagnostics and Biomedical Research:
The review covers the applications of CRISPR-based detection of target sequences, disease biomarkers and identification of genetic modifications. Specific CRISPR tools, such as Cas12- and Cas13-based systems, are suitable for use in nucleic acid detection. These methods could be used in the diagnosis of infectious diseases, the detection of genetic alterations, and gene-function analysis, as well as disease modelling, drug discovery and developing methods for experimental therapies.

Advantages and Limitations of CRISPR Technology:
In this section, the strengths of CRISPR such as programmability, ease of use, flexibility and specificity are discussed. Off-target effects, on-target unwanted edits, unpredictability of editing efficiency, immune responses and challenges in delivery are also addressed. This review compares CRISPR to other genome editing technologies, zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs) to illustrate the advantages and disadvantages of different genome editing modalities.

Delivery Systems and Safety Concerns:
This section describes ways to introduce CRISPR technology into the cell. These techniques involve the use of viral vectors, lipid nanoparticles or other delivery systems. Delivery of the genome editing system must be effective while the risk of adverse side effects is minimized, which in the case of genome editing includes off-target mutations, immune responses, off-target DNA damage, and long-term effects of genome editing. This section discusses essential laboratory studies, clinical studies, and post-treatment monitoring.

Ethics, Legal and Regulatory Issues:
This review considers ethical issues raised by CRISPR with a focus on human genome editing. It covers issues raised by somatic editing, where edited genes are limited to the individual patient, and germline editing, where edited genes may be passed on to future generations. Additional issues discussed include whether the patient has given informed consent, the safety and efficacy of the treatment, access to treatment, privacy, and potential for misuse of this technology. The review also discusses the need for appropriate regulation and responsible conduct of research before the application of CRISPR-based treatments to humans. [47,48,49]

Future Perspectives and Emerging Developments:
Future perspectives in CRISPR research, like more precise editing technologies, advanced delivery methods, minimized off-target effects and personalized gene editing and gene therapies. New therapeutic strategies are likely to broaden the treatment potential for genetic diseases and enhance molecular diagnostics. On the other hand, it remains to be seen whether the safety,

efficiency, availability and clinical utility of these novel technologies will meet expectations. [19,20,21,22,23]

- To describe how gene editing works in the molecular basis of CRISPR.
- To go through the main CRISPR systems and how do they work.Specifically, to assess the ability of CRISPR to treat genetic and other diseases.
- To compare the advantages and disadvantages of the latest genome-editing technology CRISPR with traditional technologies.

To investigate safety issues such as off-target mutations, delivery obstacles.

METHODOLOGY/LITERATURE SEARCH STRATEGY:

Databases Searched

Realist approaches are being adopted for the evaluation of complex interventions in health and social care. Programme theory development (initial ideas and assumptions about how a given intervention works) is the first step of a realist evaluation or a realist synthesis and searches of the literature can provide vital evidence for development of the programme theory. However, there is little guidance on how to search for programme theories and deciding how to search is a challenge. Using an example of identification of programme theories for a realist evaluation of Pressure Ulcer Risk Assessment Instruments in practice, the authors consider and compare different approaches to literature searches and identify important methodological issues.

Search terms and Boolean strategy:

The following keywords were combined in various sequences in order to search for scientific articles on the molecular mechanism and therapy of CRISPR, as well as its clinical use and safety.

AND OR were used to combine the above concepts to retrieve the scientific journal articles.

The search strategies were:

("CRISPR-Cas9" OR "CRISPR-Cas12") AND ("Genome Editing" OR "Gene Therapy")

("CRISPR-Based Therapeutics") AND ("Genetic Disorders" OR "Cancer Treatment") ◎("CRISPR-Cas Systems") AND ("Molecular Mechanisms" OR "DNA Repair").

("CRISPR") AND ("Off-Target Effects" OR "Safety").

("CRISPR") AND ("Delivery Systems" OR "Clinical Trials").

Inclusion and Exclusion Criteria:

Inclusion Criteria:

The following studies were included in this review:

1.Original research articles and reviews on the genome-editing technology CRISPR.

2.Research on the molecular basis of CRISPR systems (Cas9, Cas12 and Cas13, etc.) 3. Documents relating to the mechanisms of DNA repair, for example. NHEJ and HDR.

3.Review articles describing uses of CRISPR technology in cancer, other diseases, and the genetic correction of associated mutations.

4.Articles on gene therapy by means of CRISPR and clinical studies.

5.Research on the delivery modalities used, for instance, viral and non-viral delivery systems.

Articles on safety issues, off-target effects, and other drawbacks of using CRISPR.

6.Scientific articles published in the English language.

Exclusion Criteria :

The following studies were excluded from this review:

1. Articles not involving CRISPR genome editing or therapeutics.
2. Applied solely to agricultural plant and animal breeding has been an exclusion criterion.
3. Duplicate research publications.
4. Articles that lack enough detail on CRISPR mechanisms or clinical use.

Publications are not specific to the purpose and scope of the review.

5. Non-scientific and opinion journalism that is lacking in research evidence.

Study selection and data extraction:

Systematic Review of CRISPR-Based Therapeutics

1.PRISMA – Systematic search, identification, screening and inclusion of the literature.

2.PICO: Determines study inclusion based on Population, Intervention, Comparison, and Outcome.

3.Database Search: A search is performed in the following databases:Pub Med, Scopus ,Web of Science , Embase , Cochrane CENTRAL

4.Study Selection – Titles, abstracts and full-text articles are sifted.

5.Assessment of Studies for Bias: Two reviewers evaluate studies and a third reviewer makes a final decision if there are disagreements.

6.Data Collection: Relevant outcome measures are extracted.

7.Standardized: Similar inclusion and exclusion criteria are used for comparable and reliable results.

BACKGROUND:

CRISPR-Cas system classification [10,11,12,13]

The breadth of this diversity of CRISPR-Cas systems is ever growing. To covers new research, here we present a revised evolution.

Classification of CRISPR-Cas systems. The updated CRISPR-Cas classification include 2 classes, 7 types and 46 subtypes compare with the 6 types and 33 subtypes identified in our previous survey 5 years ago. In addition, a typology of the cyclic oligoadenylate-dependent signaling pathway is described for type III systems. And a newly discovered are discussed.

Alternative CRISPR-Cas functions, for example, type IV that is cleave the target DNA and the type V variants that

inhibit the target replication without cleavage. Relative abundance of CRISPR-Cas variants in genomes and metagenomes demonstrates that the systems being outlived. More prevalent, while those more recently identified are relatively uncommon. The low abundance variants are the long tail of the CRISPR-Cas apparition in prokaryotes and their viruses, and are to be characterized experimentally.

Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated protein (Cas) systems have been described as the new generation of genome engineering tools .However, their primary natural role is in bacteria and archaea through one of two mechanisms :recognizing and, in most cases, cleaving a specific sequence in the target DNA or RNA that is complementary to an individual spacer in between CRISPR repeats. The CRISPR-Cas immune response comprises three described in three main stages: adaption, expression and interference, which are then described in numerous reviews on different aspects of the molecular mechanisms of CRISPR-Cas functionality. CRISPR-Cas Systems have a characteristic modular organization that, approximately, maps onto three stages of adaptive immunity.This is what you expected of defense mechanisms – CRISPR-Cas systems are: Diverse organization of their respective genome loci, Cas gene content, domain architectures, and sequences of Cas proteins. For close to two decades since that discovery, CRISPR-Cast function10, knowledge of and a clear understanding about this the excellent system has been progressively growing by means of the mining of rapidly growing genomic and metagenomic databases. Classification for CRISPR-Cas systems authored by, to the extent possible, evolutionary associations is important to portray and additional characterization of CRISPR-cas loci in new sequenced bacterial and archaeal genomes and metagenomes and, thus, for the advancement of the entire field of CRISPR research.

The organization of CRISPR-cas loci and the Cas proteins themselves are rapidly evolving, creating a whole set of problems in the construction of a coherent and resilient classification. Three previous versions their more recent CRISPR-Cas classification and nomenclature system used a more complicated polythetic procedure. In order to Classification, compares the architecture and gene composition of CRISPR-cas loci and was paired with clustering based on sequence similarity and phylogenetic study of conserved Cas proteins, like Cas1, the Integrase enzyme that performs the central bit within the adaptation stage. CRISPR-Cas types were defined according to distinct effector modules, while subtypes were formulated in a somewhat less conventional manner, through the above criteria. A report has been described of a supplemental development recent Cas Protein Effector Database of Information and Evaluation (CasPEDIA) – The Comprehensive Classification of Class 2 Cas Enzymes based on their activity and target specificity.CRISPR-Cas New Categories Unique features of class 1 CRISPER cas systems The 2020 CRISPR-Cas system classification consisted of 6 types and 33 subtypes divided into two classes distinguish in the architectures of their effector modules directed at CRISPR RNA (crRNA) processing and interference. Since, then concerted efforts have been to locate novel CRISPR-Cas systems and to understand their evolutionary roots, which led to the identification of type VII, 13 additional subtypes and a wide variety of distinct variants . But unlike other previously identified, CRISPR-Cas systems, they are uncommon and seem to compared with the longer tail of the CRISPR-Cas distribution in prokaryotes remains to be further studied. Besides, many former uncharacterized CRISPR associated genes have been identified and the however, molecular mechanisms of some of the subtypes of CRISPR-Cas have been uncovered.

With over twenty class 1 CRISPR-Cas systems having been identified since 2020. Classification, just type VII and two subtypes, III-G and III-H, have been identified explicitly added to the classification. As we began this work on the classification update, we identified a new, divergent type III CRISPR- Cas systems, which appears to be at least subtype III-I, thus increasing the number of subtypes in class 1 to 21 (eight in type I, nine in type III, three of type IV and one of type VII).

The previously uncharacterized group type VII is exemplified by CRISPR-Cas systems broadly in several taxonomically diverse archaeal genomes and containing a metallo-β-lactamase (β-CASP) effector nuclease. The distinctive signature effector common to these systems, known as Cas14, is given its own name according to the CRISPR-Cas classification schemes, and is used to identify these loci as a new type. It is encoded in a predicted operon with Cas7 and Cas5, the subunits of the effector complicated, and for some, Cas6, which is a specialized nuclease participate in crRNA processing in other class 1 systems. Type VII loci do not have repeats and adaptation modules in associated CRISPR array have multiple examples of substitutions suggesting that the an array are more do not frequently incorporate new spacers.Remarkably, the Cas14 protein has a carboxy-terminal domain with a structure similar to that of the β-CASP domain as well as the β-CASP domain C-terminal of Cas10, the large subunit of the type III effector module, suggesting an evolutionary connection between Nexus of high-tech roots types III and VII. It is also based on the feedback link of Dentical similarity between the Cas5 proteins of subtypeIII-D9 and typeVII. The Cas7 catalytic residues needed to target RNA cleavage by type III systems are not conserved in type VII.

Instead, type VII systems have been demonstrated to target RNA through a crRNA-dependent pathway, which cleaves the target RNA by Cas14 using its nuclease activity. Type VII systems are believed to be simple and likely derived from type III through the reductive pathway . Nevertheless, a recently elucidated cryogenic-electron-microscopy structure of the type VII effector complex is made up of 12 or more subunits. – Cas14 is attached to the Cas7 backbone through the support of it's along with the Cas10 remnant domain, and this specific complicated one of the largest of the class 1 systems.

The three previously unknown types of type III CRISPR-Cas systems-that is, III-G (Sulfolobales-specific, previously reported as unclassified, III-H (some archaea and a few bacteria) metagenome-assembled genomes (MAGs)) and III-I (detectable in more than 160 genomes in the National Center for Biotechnology Information

(NCBI) non-redundant (NR) database, primarily from the phyla Thermodesulfobacteriota and Chloroflexota)- are not closely related but share certain features indicative of reductive evolution. For subtypes III-G and III-H, carried within the polymerase/cyclase domain of Cas10, Inhibited, i.e., the large subunit of the effector complex by the replacement of the catalytic amino acids. The loss of ability to production of cyclic oligoadenylates (cOAs).

The deficiency of and the stimulation of genes encoding additional proteins that carry a domain for binding cOA (e.g.CRISPR-associated Rossmann fold (CARF) or SMODS (second messenger oligonucleotide or dinucleotide synthetase)-associated and fused to different effector domains (SAVED)), attached to an effector domain, such as higher eukaryotes and prokaryotes nucleotide-binding (HEPN) Rnase or other effectors that are typical of type III CRISPR-Cas systems. As a result, the subtypes have been removed from cOA search induces collateral Rnase activity in most type III systems. And though subtype III-H is evolutionarily distant from III-F but a characteristic of III-H systems is a distinctively diverged small subunit (Cas11) which seemingly has substituted the CAS10 gene with the C-terminal part of Cas 10. Whereas, in subtype III-G, Csx26, which is the hallmark protein of this subtype, could supplant Cas11. Both these novel subtypes have no adaptation modules and no CRISPR array in the effector complexes has exclusively been detected in III-G loci, indicating that this system co-opts CrRNAs from other CRISPR-Cas loci in trans. Due to absence of conservation in the catalytic aspartates of Cas7 and the existence of seemingly active HD-nuclease domains in Cas10, both III-G and III-H are predicted to split DNA targets.The effector module of the new subtype III-I system identified in the present study possesses two distinct features: (1) an exceedingly diverged Cas10 lacking the amino-terminal polymerase/cyclase domain; this protein is not sequence-related to Cas10 that we have detected, but confidently identified as Cas10 homologue in the structure similarity search distance-matrix alignment (DALI Z-score = 10.9) and 2) A multidomain protein with a domain organization akin to Casi, that of Cas7-11, the effector protein of subtype III-E but apparently originating separately from an alternative subtype III-D. The III-I effector protein is made of three fused Cas7 domains and a Cas11 domain that is missing the N-terminal Cas7 domain; and an insertion in the C-terminal Cas7, this insertion is present in Cas7. On the basis of the presence of conserved aspartates within each of the three Cas7 domains of the mono or multi-subscribed most likely cleaves RNA. We will use to denote the III-I effector Cas7-11i and therefore correctly change the name of for the III-E effector to Cas7-11e.In addition to type VII and the three previously uncharacterized subtypes, several variants of class 1 CRISPR-Cas systems with distinct HNH nuclease in related domain architectures and functional variants have been found in recent studies. Three variants (I-E2, I-F4 and IV-A2) also contain an HNH nuclease, which is found in fusions with Cas5, Cas8f and CasDinG proteins respectively. Robust crRNA-guided double-stranded DNA (dsDNA) but cleavage activity has been shown for all of these variants notably, Cas3 helicase-nuclease is usually absent from I-E2 and I-F4 variants, is the part of the T cell that cleaves the target DNA in most type I CRISPR-Cas mechanisms, so that the HNH nuclease appears to take the place of the nuclease activity of the HD-nuclease domain of Cas3. The HNH nuclease is most often contained within an MGE, which encode group I self-splicing introns. Cas9, the type II effector also has an HNH nuclease that has been inserted into the other RuvC-like nuclease domain of Cas9 and defines the target DNA strand cleaves. The nucleotide for the new HNH-encoding variants reveals that this mobile nuclease has been independently acquired in various CRISPR-Cas systems. In contrast, in the case of Cas9 the HNH nuclease cleaves only one DNA strand within the dsDNA target, in the novel variants HNH is the lone effector nuclease that cuts both strands. The IV-A2 variant stands out.The first type IV system in which the target was cleaved. All other type IV-A and IV-B systems, which do not have effector nucleases, seem to be inhibited the reproduction of MGE using to inhibit transcription, at the time of itself to be the topic of a question in when IV-A9. Type IV-C systems are expected to cleave DNA through the HD domain but have not yet been characterized experimentally. Another new variant, I-E3, includes a different nuclease of the family PD-(D/E) xK that is fused to Cas119. Several of these systems are associated with Cas3, such as a seemingly active HD-nuclease domain, then the function of the PD-(D/E)

xK nuclease remains to be determined. Another common theme in the evolution of CRISPR-Cas is the recruitment of these systems by large transposons, which allow RNA-guided transposition. The CRISPR-associatedTn7-like transposons (CASTs) of subtypes I-F and I-B. In the previous classification such as I-F3 and I-B2, respectively. Most recently, three other CAST subtypes that have that, from B- I-D, from B- I-C and IV-A20-22 and a I-A system was identified linked to Mu-like transposons. Unlike most CASTs in which the Cas effectors of the CRISPR-Cas are made to lose their activity the others I-D CASTs appear to be active and may be the latest transposon-CRISPR-Cas systems to have evolved, a dual function or as a genuine CRISPR –CAST. Or as a bona fide CRISPR-Cas system. The I-A CASTs identified with Mu-like transposons have not have not yet been experimentally characterized and are presumed to interact which differ from the way the transposition machinery is because these transposons do not contain TniQ-TnsD protein which is a requisite for this process of all the previously identified CASTs. Lastly, a unique CAST type (I-E5), which also lacked TniQ-TnsD, has been recently identified adjacent to a specific group of telomeric transposons. This CAST includes an active the HD-nuclease domain within the Cas3 protein potentially serving as a double function. The other type of CASTs except for subtype IV-A which I so far only one , for example, previously found, were added to this group as variants of the respective CRISPR-Cas subtypes.Range of sensors and effectors of type III systems:

The vast majority of type III CRISPR-Cas systems are orders of magnitude more complex than others. The largest of these types including a gorgeous variety of accessory proteins. In the revised classification, type III is subdivided into nine subtypes, each of which includes a

fixed complement of core effector genes and constitutes a separate clade within the Cas10 phylogenetic tree. The other exceptions being subtype III-E lacking a cas10 gene and subtype III-I, with its highly diverged Cas10 derivative. However, loci architecture comparison and phylogenetic analysis of Cas7 types clearly indicate that III-E and III-I are from different variants of subtype III-D28.The diversity of type III CRISPR-Cas systems cannot be captured by the division into subtypes and variants more subtle approach. Signalling built into the core of type III pathway by which, on antigen identification, the polymerase/ the cas10 cyclase domain produces cOA or S-adenosyl methionine(SAM)-AMP, which are second messengers that are fed by the sensor domain of an auxiliary protein. Connecting with the messenger molecule induces a conformational change in the ancillary protein, producing a activation of its effector domain, which results in growth arrest. Moreover, these signal transduction systems are also being suppressed through either ring nucleases degrading cOA or by enzymes responsible for cleavage of SAM-ATP32. Sensors, effectors and ring nucleases among these pathways are present in various forms resulting in combinatorial diversity. Although the polymerases/cyclase domain of Cas10 is sometimes deactivated(subtypes III-C,III-G and III-H) or were lost (subtype III-I) so that about 97% of the Cas10s are thought to be active and messenger-producing.The cOA sensors of type III systems are nearly all CARF and related SAVED domains. While many ring additionally, nuclease have CARF domains and a small number of ring nuclease have other enzymatic domains distinct from CARF have also been identified. The classification of its CARF domains by sequence identity resulted in 11 families, seven of which were composed largely of proteinsthen containing a sensor (CARF) and an effector domains, three include known or predicted ring nuclease and one (Csm6) composed of proteins seemingly fulfilling all three roles. We now define a systematic nomenclature for the major categories of sensor and ring nuclease CARF domain-containing protein to enable the systematic analysis and characterization of type III CRISPR-Cas systems and the interactions between them. To facilitate comparison with and the published work, legacy names will also be displayed if present. All experimentally characterized families ring nuclease types are currently numbered as Crn1 through Crn5, with associated legacy designations also listed retained.The dominant cOA-activated effectors are HEPN Rnases but a number of additional effectors have been described. They include Rnases of PIN family and RelE, Dnases of the PD-(D/E)xK and HD families, proteases of the Caspase and Lon families, some other enzymes in addition to transcriptional regulators and also proteins. Whereas the cOA pathway is well-established, certain steps of the SAM-AMP pathway have not been sufficiently; membrane protein related to the magnesium transporter CorA found in some III-B and III-D loci apparently to detect SAM-AMP through its own sensor domain appears to be a trail of this avenue. But, it is still unsure whether the CorA homologue in III-D systems detects SAM-AMP/another signaling molecule, especially since, unlike the III-B loci, established SAM-AMP cleavage enzymes are encoded elsewhere outside of the III-D loci. Another interesting clade present in the Cas10 tree is the V-A2 version has no visible Cas11, and Cas10 in this. That are predicted to be synthesis cOA, which some systems likely directly by NucC the nuclease effector34. Specifically, the conservation of the catalytic aspartate in Cas7 indicates this system could be targeting both RNA (via Cas7) and DNA (via NucC).Sensors and effectors are distributed throughout the clades of the Cas10 tree, indicative of a high degree of module shuffling and horizontal gene transfer arranging of the cOA and SAM-AMP eliciting pathways in type III systems. Despite the profound rearranging of components of the signalling pathways some trends to note are: Were furthermore noteworthy. First, ring nucleases are an important part of the destroyed by the secondary signalling pathway and are found in nearly all loci encoding enzymatically active Cas10 for which the cOA synthesis activity is intact. Second, SAM-AMP is degraded by different enzymes, encoded in the corresponding loci.

Analysis of anomalies from these two trends may uncover Cas10s that make other messengers, as well as alternative sensors and nucleases.Detection of many type 2 CRISPR-Cas systems. The identification of many type 2 CRISPR-Cas systems, mostly variants of type V, was the Hallmark of the 2020 update of the CRISPR-Cas classification. A unique attribute of this class 2 CRISPR-Cas system is their effector machinery includes a single multidomain protein, namely Cas9 in type II, Cas12 in type V and Cas13 in type VI. Class 2 CRISPR-Cas systems are, structure, less compact and more loosely arranged than the cells of class 1 and classification is by mainly on comparison and phylogenetic analysis of the single large effector signature proteins. The identification of additional variants in recent years has resulted in the subdivision of subtype II-D. This subset has the former II-C2 variant with small archaeal Cas9s8, designated II-D in CasPEDIA13, together with the newly described alternate including the tinyest known Possibly ancestral cas9s as well as based II-D37.

Notably, II-D systems are prevalent in symbiotic and parasitic nanoarchaea of the Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaeota (DPANN) superphylum, along with some bacteria, but that formerly class 2 systems were almost exclusively bacterial and were absent from the archaea. It has been demonstrated that, like all type II CRISPR-Cas systems, type II-D loci encode trans-activating CRISPR

RNA (tracrRNA).Type II and V: tracrRNA mediates crRNA maturation and interference. In certain Type V system, however, Cas12 cleaves pre-crRNA, suggesting a different origin of tracrRNA in Type II and V.

Type VI-E and VI-F: These are two newly discovered types. Cas13e, the smallest and most compact Cas13 protein to date, has been referred to as the latest Cas13. The VI-F is currently only known to exist in bacteria of the bacterial genus Brachispira.

Origin of Cas13: Structural analyses indicate that the Cas13b was unique and may have been derived separately from other Cas13 effectors. Among the Type

VI-B effectors, the smallest are grouped into variant VI-B3.

Accessory proteins: Class 2 CRISPR-Cas systems can include different accessory proteins to induce a variety of immune responses.

Type V subtypes: The rare subtypes including V-C, V-E, V-G, V-H, V-I, V-J, V-L, V-N, V-O and V-P are detected mostly in these data sets.

Classification challenges: Some peculiar Class 1 systems (e.g., those related to MGEs and HNH nucleases) need to be classified by computationally and manually.

Mechanism of genome editing (DSB, NHEJ, HDR) [4,5,6,8,9,10]

The gene editing technologies using programmable nuclease Cas9/gRNA consists of the generation of DSBs at the target site of genomic DNA in the first step. In mammals, two repair pathways, nonhomologous end joining (NHEJ) and homology-directed repair (HDR), are identified to repair these DSBs, and the outcome of Cas9 mediated gene editing depends on the ratio of NHEJ and HDR, which are competing with each other. Although HDR is desirable, favoring high-fidelity repair, it is biased in the biological world. Mammalian cells tend to use the NHEJ pathway than the HDR pathway via several mechanisms: NHEJ is active through all cell cycles, while the HDR pathway can operate only during S/G2 phase; NHEJ is faster than HDR pathway; NHEJ suppresses the HDR pathway. Thus, we predict that the outcome of programmed DNA lesioning could be precisely controlled by manipulating the pathway choice of DNA repair. In this review, we introduce the repair pathways of DSB, mechanisms involved in the choice between these pathways based on DNA resection, and advancement in the research to develop a strategy to increase the HDR frequency in mammalian cells based on manipulating pathway choice of DNA repair. We also discussed remaining problems on improving HDR efficiency. We hope that this review helps in the development of the CRISPR/Cas9 technology for precise gene editing.

NHEJ (Non-Homologous End Joining) is a form of DNA repair which closes DSBs (double-strand breaks) in DNA using available DNA ends without involving a homologous template.

Mechanism:

Ku70-Ku80 binding: Detects and attaches to broken DNA ends and prevents severe DNA-end resection.

53BP1 recruitment: Protects the DNA ends and blocks the initiation of BRCA1-dependent HDR.

DNA-PKcs Recruitment: Assists in aligning broken DNA ends as well as coordinating their processing.

Terminal processing: Artemis trims excess nucleotides, whereas DNA polymerases Pol μ and Pol λ introduce small patches of nucleotides when needed.

Final ligation: XRCC4 and DNA ligase IV complete the joining of the DNA ends.

NHEJ in CRISPR-Cas9 gene editing: [8,10,20,27]

Cas9 creates double-strand breaks in DNA.Multiple rounds of cutting may lead to short insertions or deletions (indels). Indel mutations can disrupt or inactivate the target gene.NHEJ is active throughout the cell cycle and is a fast repair pathway.While NHEJ can repair DNA accurately in some instances, it is more random than HDR and thus less reliable for editing genes.

Homology-directed repair (HDR) corrects a DSB by using a homologous (e.g., sister chromatid or donor DNA) as a template to re-establish the correct DNA sequence.

Mechanism:

Double-strand break (DSB) Both pathways, cutes in during S and G2 phases of the cell cycle, where it has the availability of a sister chromatid for repair.

DNA end resection The MRN complex (MRE11, RAD50 and NBS1) detects the break and assists in ATM signalling. It attracts CtIP and starts DNA end processing. Exo1 or the DNA2-BLM complex further process the DNA end, generating a 3 single-stranded DNA (ssDNA) overhang.

RPA binding and RAD51 loading RPA attaches to the exposed ssDNA to protect and stabilize it. BRCA1, BRCA2, and PALB2 facilitate replacement of RPA by RAD51 to form a nucleoprotein filament on the ssDNA.

Homology search and strand invasion RAD51 begins to look for another match of DNA sequence in the donor DNA or sister chromatid. The 3 of the ssDNA dissociates into the complementary and same strand of the homologous duplex DNA.

DNA synthesis, resolution The invading 3′ end serves as primer for DNA synthesis with the donor template. The repair intermediates have been processed via various HDR pathways that may include one or two Holliday junctions. The junctions are resolved by resolution enzymes.

Cas variants: Cas9, Cas12, Cas13 [13,14,15,16]

Here we review the development of CRISPR-Cas9/Cas12/Cas13 nucleases, DNA base editors, prime editors, and RNA base editors, working on their editing accuracy and the evaluation and correction of safety, the limits of editing specificity and efficiency. We summarize the tools from DNA editing to RNA and the capabilities and limitations of each CRISPR tool and underline the opportunities for future improvements and applications for basic research and the clinical and therapeutic perspectives for their use in patients.

Class 2 CRISPR-Cas systems have been identified, that is type II, type V and type VI along with their effector proteins. Cas9, Cas12, and Cas13, respectively of them, most Cas9 and Cas12 variants and the, Cas13 variants show preferential RNA targeting and cleavage activity. Cas9 nucleases are directed to the target site by single guide RNAs (sgRNAs) that were constructed by combining the CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA) into a single RNA; usually this causes a blunt-ended double-strand break (DSB) at the protospacer sequence. When the R loop has been formed by the Cas9-guide RNA ribonucleoprotein complex the HNH-nuclease domain makes cuts in the target DNA strand that is bound by the guide RNA, where the RuvC-like nuclease domain cuts the PAM-containing nontarget DNA strand 3 bp upstream of the

PAM, where the RuvC-like nuclease domain cleaves the PAM-containing nontarget DNA strand 3 bp upstream of the PAM, Cas9 can also generate stagger cutting that resulting predictable 1-bp Insertions. Unlike Cas9, a number of the Cas12 nucleases are naturally RNAguided by a single crRNA. Further, Cas12 nucleases have only a few a single RuvC-like nuclease domain responsible for cleaving both target DNA strands, staggered DSB cuts within regions of the protospacers distal to the sequence the protospacers are distal to the PAM.

The Cas9 protein has six domains that are: REC I, REC II, bridge helix, PAM-interacting, HNH, and RuvC. The role of REC I domain is to bind the gRNA, the role of REC II is unknown, bridge helix is to aid in the initiation of DNA cleaving, PAM-interacting domain to bind to the protospacer adjacent motif and HNH and RuvC domains are responsible for cleaving the DNA.

Cas9 without gRNA is inactive. Cas9 is activated by designing sgRNA, a single RNA sequence in which crRNA and tracrRNA are joined together. Complementary base pairings between gRNA and the target DNA sequence determine the specificity of the target. Streptococcus pyogenes Cas9 (SpyCas9) recognizes the PAM (5-NGG-3) sequence and induces a double-strand break (DSB) on the DNA strand about 3 bp from the PAM. The target strand is cleaved by the RuvC domain while the non-target strand is cleaved by the HNH domain.

The Cas12 protein needs the crRNAs alone in order to get a efficient cleavage of ssDNA and dsDNA.

Cas12 protein has the RuvC and the nuclease lobe (NUC) domains for cleavage activity. Similar to Cas9, Cas12 finds a possible target site next to a PAM sequence. After Cas12 begins to find, it begins to R-loop, it forms base pair hybridization between the crRNA and the target DNA strand. And also measured during this step, Cas12 complementary to the<17 bp of the target sequence and results in an R-loop formation. Once R-loop is formed, and the Cas12 protein cleaves with its active RuvC domain the non-target strand in the presence of the PAM sequence, the activity of the RuvC domain. Possibility that the Cas12 protein may not be able to cut the target DNA strand, yet clearly studied.Cas13a protein can be activated with single crRNA, processed from pre-crRNA. It consists of NUC lobes and a two-domain HEPN Rnase structure that involved in targeting and cleaving RNA. LshCas13a usually detects a complementary target sequence that consists of 22-28 nucleotides within single-stranded RNA (ssRNA). The presence of the target sequence next to the protospacer-flanking site (PFS) in the RNA can further guide the process and often prefers Adenine (A), Uracil (U), or Cytosine (C) at the 3′ end. There is no requirement of tracrRNA for Cas13a cleavage and Cas13a makes it complex with crRNA to target the RNA sequences.

Cas13b provides more accurate target recognition than Cas13a. It binds to RNA targets with specific flanking sequences containing A, U, or G bases at the 5 end and NAN/NNA motifs at the 3 end. The Cas13b-crRNA complex targets an RNA and induces conformational changes that result in cleavage of the target ssRNA. Its mechanism of action is still not well understood, but Cas13b has also been explored as a tool for RNA editing.

Delivery platforms Viral vectors (AAV, lentivirus) Non-viral vectors (LNPs, nanoparticles, RNP) [21,22,23,24,46]

Ex vivo versus in vivo approaches

Successful delivery of the CRISPR/Cas9 system has attracted a lot of attention. Multiple methods are used to deliver CRISPR/Cas9 including physical methods, viral vector-based approaches, and non-viral vector-based approaches. These methods have their pros and cons. Off-target effects should be minimized and more efficient and safe delivery methods need to be developed.

Lentiviral Vectors (LV) LVs of about 7 kb size carrying the SpCas9 gene + sgRNA are capable of reaching its target. Third-generation self-inactivating (SIN) lentiviral vectors are a measure of improved biosafety. Though they can deliver CRISPR/Cas9 very well, targeted delivery has proved to be a hassle. [21,22,23,24,46]

Researchers have used pseudo typed lentiviral vectors to deliver targeted delivery and gene mutation. Researchers have also used non-integrating lentiviral vectors for sickle cell disease (SCD) mutation correction without the long-term risk of genetic damage at high multiplicities of infection (MOI). These vectors are difficult to produce.Adeno-Associated Viral Vectors (AAV) is popular for delivering CRISPR/Cas9 because it can be targeted to specific tissues, and is less likely to integrate into the genome. It has been explored for treating a variety of genetic diseases, including phenylketonuria and Rett syndrome. The ability of AAV to sustain gene expression might be advantageous, but longer Cas9 expression could result in higher levels of off-target editing, toxicity, and immune responses. Researchers have engineered

Self-eliminating AAVs in which the Cas9 removes the vector DNA after editing is performed, but immune responses to AAV and Cas9 are still major issues.Adenoviral Vectors (AV) have the capacity to carry ~8kb of genetic material, high capacity adenoviral vectors (HCAdV) up to 35 kb. They are also suitable for delivery of multiple components of the CRISPR system due to their high capacity and low risk of genome integration. Adenoviral CRISPR delivery has been explored for genetic diseases such as hemophilia B and for disabling hepatitis B virus DNA. One vector can carry Cas9 and multiple guide RNAs (gRNA). They are limited by immune responses, toxicity to cells, and unintended off-target editing.

Non-Viral Vectors: Non-viral vectors also do not use viruses to deliver CRISPR/Cas9 systems. Common examples are polymer nanoparticles, lipid nanoparticles (LNPs), and dendrimers. These can be used to carry either Cas9 protein or sgRNA, mRNA or plasmid DNA, and have comparable advantages to virus-based vectors such as low immunogenicity. Types of non-viral vectors, which include LNPs for the delivery of Cas9 mRNA and sgRNA, as well as polymer nanoparticles, for example, which can enhance cellular uptake and controlled release of the tools can be developed for the treatment of cancer and genetic disease. The efficiency of delivery, specificity and penetration into cells are some of the areas of development.Methodology

Ex Vivo with CRISPR/Cas9: [21,22,23,46]

The cells are taken out of the patient's body and their genes are edited in the lab, often with CRISPR / Cas9. Take the cells out of the body – and send the CRISPR / Cas9 system into the cells, where it can mutate or correct the gene of interest. Then culture the cells to check that the gene was successfully changed and is safe before returning to the body. Benefit: The cells can be checked before they are given to the patient. Example: Gene editing of blood stem cells to treat sickle cell disease and beta-thalassaemia.

In Vivo Approach in CRISPR/Cas9

In vivo means "in the living", which involves injecting the CRISPR/Cas9 directly into the patient in order to edit genes in the target cell. This is achieved through the use of viral vectors such as AAV or non-viral vectors, such as lipid nanoparticles that transport Cas9 and guide RNA directly to the tissue of interest. Once it's in the cell, it gets in the DNA, recognises the target sequence and then modifies the gene. This method enables tissues that are hard to be removed to be edited outside the body, but targeted delivery, immune responses and unwanted editing are some obstacles. Example: Modification of PCSK9 gene (target for high cholesterol) in liver cells.

CURRENT EVIDENCE [30,31,32,33,34,35,36]

Introduction and mechanism:

CRISPR (clustered regularly interspaced short palindromic repeats) is a common, programmable,genome editing technology. To cut the DNA, a guide RNA (gRNA) is used to find the target DNA sequence next to a protospacer-adjacent motif(PAM), which is then cut by a Cas enzyme (often Cas9). This double stranded DNA is repaired through either non-homologous end joining (NHEJ) that can create insertions or deletions (indels) or through homology-directed repair (HDR) which can insert a custom sequence from a supplied repair template. This technology has gone from bench to bedside with use in clinical trials and some FDA approved treatments for specific inherited blood diseases.

Delivery platforms and editing approaches

Delivery vehicles for the editing instrument can include plasmid DNA, Cas9-gRNA RNP ,nanoparticles or viral vectors.RNP improvides the active protein and guide and is a transfecting agent in vivo. Lipid, polymer and gold-based nanoparticles are used ,with Lipid nanoparticles (LNPs)studied extensively for delivery to the liver. Viral vectors (adenovirus, lentivirus, adenovirus associated virus (AAV) have distinct characteristics of tissue targeting, payload, duration, immune response and cost.

- Ex vivo editing : patient cells are removed, edited,grown up and quality tested outside the body,then infused back. This enables personalisation but is sophisticated and expe.
- In vivo editing : components are delivered in the body,for instance intravenously or locally/intravitally. This could make treatment less complicated but tissue-delivery and regulation of editing are challenging.
- Base editors change specific bases withkut causing double strand breaks and prime editors employ a Cas nickade,reverse transcripts,and other guide RNAs. Dcas9 (dead Cas9) can repress or activate gene expression (CRISPRi/CRISPRa) and Cas13 can cut RNA.

Major clinical applications [30,31,32,33,34,35,36,37,38,39,40,41]

The most advanced clinical candidate in ex vivo editing of autologous CD34 + hematopoietic stem and progenitor cells for sickle cell disease (SCD)and transfusion-dependent beta thalassemia (TDT). Exagamglogene autotemcel (exa-cel;Casgevy)targets the erythroid enhancer of BCL11A thereby inhibiting its ability to suppress fetal haemoglobin (HbF). This results in high levels of Hbf, lessening red-cell sickling and ameliorating ineffective erythropoiesis. Cells are reintroduced following myeloablation conditioning. In vivo liver-directed programmes are:

NTLA-2001 for TTR amyloidosis and NTLA-2002 for hereditary angioedema via KLKB1 is in development;lipid disorders including PCSK9, ANGPTL3 and LPA; and CEP290 editing in Leber congenital amaurosis type 10. Cas9-engineered T cells are under investigation in cancer immunotherapy.

CLINICAL APPLICATION:

Cancer immunotherapy and cellular engineering [34,35,36]

CRISPR can modify T cells to improve chimeric antigen receptor (CAR)-T therapy. Targets include TRAC (reducing endogenous T-cell receptor activity), B2M (altering HLA class I expression), CD52 (supporting resistance to selected lymphodepleting treatment) and immune checkpoints PD-1, CTLA-4 and LAG-3. Other targets, such as CD7, GM-CSF, Regnase-1 and TGFBR2, are studied to improve activity in suppressive tumour environments. Multiplex editing may enable allogeneic 'off-the-shelf' CAR-T cells. Important risks include host rejection, graft-versus-host disease (GvHD), cytokine-release syndrome (CRS) and T-cell exhaustion.

Off-target effects and genomic consequences [25,26,27,28,29]

Off-target editing occurs when Cas is directed to a DNA sequence similar to, but different from, the intended target. Mismatches are often more tolerated in PAM-distal regions than in the PAM-proximal seed region. DNA or RNA bulges may also permit unintended binding. Unwanted cleavage can cause small indels or larger structural variants (SVs), including deletions, insertions, inversions and translocations.

Large deletions may remove coding exons or intronic regulatory regions, alter gene expression, create gene fusions or cause loss of heterozygosity (LOH). Extensive chromosome fragmentation followed by abnormal repair can produce chromothripsis. These events may impair cell function or favour clonal expansion. Double-strand-break stress may select cells with defective TP53 signalling, potentially increasing malignant transformation risk.

Manufacturing and economic challenges

Autologous ex vivo therapy requires individual cell collection, transport, editing, expansion, quality testing, release and reinfusion. Manual workflows and long culture periods increase turnaround time and batch variability. GMP-grade Cas nucleases, synthetic gRNAs, viral vectors and quality-control assays add cost. Viral-vector manufacture is expensive and limited by packaging capacity and complex production; in vivo delivery is also restricted by tissue tropism and payload constraints.

Total treatment cost includes more than the gene-editing product: cell collection, conditioning chemotherapy, hospital admission, supportive care, fertility preservation and long-term monitoring all contribute. Casgevy's reported US wholesale acquisition price of about US$2.2 million illustrates the financial burden; actual costs and access differ between health systems.

SAFETY AND LIMITATIONS:

Durability, persistence and follow-up

Durability depends on the edited cell type, tissue turnover, delivery system and persistence of therapeutic cells. In ex vivo autologous haematopoietic stem-cell therapy, long-term repopulating cells can engraft after conditioning and pass the edit to daughter cells. This can maintain HbF production and provide a donor-independent effect without GvHD. Continued follow-up is required to confirm benefit and detect late complications.

Allogeneic CAR-T cells may show reduced persistence because of host rejection, exhaustion or loss of function. Multiplex editing of TRAC, B2M, PD-1, Regnase-1 and TGFBR2 is being investigated to reduce immune recognition and improve persistence; TGFBR2 is being investigated to reduce immune recognition and improve persistence; durable clinical benefit still requires confirmation.

In regenerative tissues such as skeletal muscle, durability is more difficult. AAV may not efficiently edit quiescent satellite stem cells; during muscle regeneration, unedited cells can dilute edited nuclei and reduce sustained dystrophin expression. Neutralising antibodies against viral capsids may prevent repeat dosing. LNPs offer non-viral delivery but extrahepatic targeting remains challenging.

Because off-target effects, chromosomal rearrangements, clonal expansion and oncogenesis may appear late, regulators may require surveillance for up to 15 years, depending on product risk. Monitoring can include clinical review, blood counts, clonal population analysis, malignancy surveillance, vector-related assessment and measurement of continued therapeutic effect.

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Durability and delivery: key platform differences

- Autologous HSCs: potentially long-lasting through stem-cell engraftment; monitor stable therapeutic response and clonal expansion.
- Allogeneic CAR-T: persistence may be limited by rejection and senescence; monitor cell kinetics and secondary T-cell malignancies.
- AAV in vivo: durability depends on target-cell longevity; monitor transgene activity and late immune toxicity.
- Non-viral LNPs: useful for transient delivery, especially to liver; assess phenotype maintenance, off-target effects and tissue distribution.

Ethics, equity and access

Somatic editing changes non-reproductive cells and is not intended to be inherited. It may be ethically acceptable when supported by informed consent, a favourable risk–benefit assessment, safety monitoring and fair access. Germline editing changes embryos, gametes or early developmental cells and may pass alterations to future generations. Concerns include off-target mutations, large chromosomal changes, mosaicism, uncertain developmental effects and the inability of future individuals to consent. Potential enhancement and eugenic uses raise further concerns; clinical heritable human genome editing remains widely restricted. [47,48,49]

Access is unequal because therapies are expensive and require specialist facilities, trained staff, transplant services and long-term follow-up. Low- and middle-income countries may carry a high burden of conditions such as SCD but lack the infrastructure for complex cell processing. Reimbursement and insurance gaps add delays and may deepen health and social inequalities.

- Potential solutions include automated and standardised manufacturing, shared GMP reagent supply chains and regional treatment centres.
- Validated allogeneic cell platforms may reduce patient-specific production time and cost, although immune compatibility and safety must be addressed.
- Non-viral delivery and distributed or point-of-care manufacturing may reduce vector and transport costs, provided consistent quality control is maintained.

Regulation and international oversight

Regulatory requirements vary by country. The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) assess gene-editing products through biologics and advanced-therapy frameworks. Review covers product identity and potency, GMP

manufacturing, preclinical safety, clinical evidence, informed consent, traceability and post-treatment surveillance. In the United States, gene therapy products are reviewed through FDA biologics pathways, including the Center for Biologics Evaluation and Research (CBER); in Europe, advanced therapy medicinal product (ATMP) requirements apply. Expedited pathways may support serious diseases but do not remove the need for robust safety evidence. [45,46,47,48,49,50]

The 2018 report of implantation of genome-edited human embryos in China was widely condemned and exposed gaps in international oversight. Strong laws, independent ethics review, transparent reporting and international cooperation are needed to prevent unsafe clinical use and medical tourism.

Milestones and future directions

Key milestones include the description of CRISPR repeat sequences in 1987, demonstration of programmable Cas9 editing in 2012, early clinical programmes in the late 2010s and regulatory approvals of exagamglogene autotemcel for SCD and TDT in 2023–2024. Other programmes have explored liver, eye, immune-cell and neuromuscular targets. Trial-stage programmes should not be confused with approved treatments. Trial-stage programmes should not be confused with approved treatments.

EMERGING ADVANCES:

The 6 areas described in the literature constitute a logical continuum of: DNA editors with high precision (base and prime editing), other than DNA editing modalities (epigenome and RNA editing), the Cas variants powering these technologies, guide-design through artificial intelligence (AI), and delivery methods carrying it into cells. The included literature consists mainly of reviews and only a couple of original papers, therefore the figure shown below reflects the state-of-the-art from this body of work rather than an overarching consensus on CRISPR-based genome editing research.

Base Editing & Prime Editing : [17,18,19,20,43,44]

Base editors combine the catalytically inactive Cas nuclease with a deaminase to edit a DNA strand without creating double-stranded breaks (DSBs)

. Cytosine base editors (CBE) convert C•G to T•A, and adenine base editors (ABE) edit A•T to G•C mutations, and cover all four transition mutations

. Advanced architecture allows editing transversion (C to G) mutations and dual editors.

Prime editing combines a Cas9 nickase and a reverse transcriptase with the help of pegRNA, allowing all substitutions as well as small insertions/deletions.

Both base and prime editing methods have lower levels of untargeted indel formation compared to Cas9 nuclease activity, especially in non-proliferative cells.

One notable exception – more indel formation by CBEs in comparison to ABEs , as well as off-target activity of cytosine editors without guide RNA's.

Epigenome Editing & RNA Editing : [14,15,16,43]

Epigenome editors use the DNA-targeting platform (CRISPRi/CRISPRa) to modify the gene expression through heritable and reversible modifications without breaking the DNA.

Combination of the dCas9 with effectors like KRAB or TET3 enables to achieve programmable repressor or activator

, with a variety of applications from gene regulation to cellular programming.

RNA editing through Cas13 is performed by using RNA-guided RNA endonuclease :

It was used for neutralization of SARS-CoV-2 replication by targeting viral RNA and there are multiple reviews comparing Cas13 methods to RNA interference/antisense oligonucleotides

High-Fidelity Cas Variants & AI-Assisted [24,25,26]
Guide Design:

Engineering of the Cas variants (eSpCas9, Cas9-HF1, HypaCas9, EvoCas9, Sniper-Cas9) minimizes off-target mutations through guided or directed mutation, but it usually reduces on-target.

AI-Assisted Guide RNA Design [20,25,26]

Artificial intelligence (AI) is becoming an important tool in improving the accuracy and efficiency of CRISPR-based gene editing. Designing an effective guide RNA (gRNA) is essential because it determines where the Cas enzyme makes changes in the DNA. AI-based computational models analyse DNA sequences and predict suitable guide RNAs based on their targeting efficiency and specificity. These tools also help identify potential off-target sites, reducing unwanted genetic changes. Machine learning and deep learning approaches further support the selection of effective guide RNA sequences, making CRISPR technology more precise and reliable.

Targeted LNP and Next-Generation Delivery Systems :

The successful application of CRISPR technology depends not only on accurate gene editing but also on delivering its components safely into target cells. Lipid nanoparticles (LNPs) are widely studied delivery systems that can carry CRISPR components, such as Cas9 mRNA and guide RNA, into cells. Targeted LNPs are designed to deliver these genetic materials to specific tissues or organs, improving delivery efficiency while reducing unwanted effects in other cells. Next-generation delivery approaches, including engineered viral vectors, polymer-based nanoparticles and extracellular vesicles, are also being explored. These advanced systems aim to improve tissue targeting, cellular uptake, safety and overall therapeutic effectiveness. [21,22,23,46]

Pharmacist's role:

Patient Education and Counselling about Gene Therapies :

The pharmacists inform the patient about the gene therapy, its need, advantages, risks, and so on. They help

the patient understand the process of treatment and how to follow the doctors' advice. [30,31,32,45,46]

Handling, Storage, and Dispensing of ATMPs : [30,31,32,45,46] Advanced therapy medicinal products (ATMPs) have specific requirements for handling and storage. They are managed to guarantee the correct storage temperature and prevent deterioration throughout transportation, storage and dispensing.

Medication Safety and Pharmacovigilance :
Pharmacists can use this information to report safety-related issues, as well as contribute to the safe use of gene therapies. [25,26,27,28,29,45,46]

Drug Interactions and Supportive Care Interactions potential of gene therapies with other medications are detected by pharmacists and the expected interactions are summarized for patients. Patients are also informed about adjunct medications, such as conditioning schedules and immunosuppressants, if necessary. [30,31,32,45,46]

Involvement in Multidisciplinary Clinical Teams :
If you become a pharmacist, you will work with doctors, nurses and other healthcare professionals. Your expertise with medicines can also help to ensure you choose the right treatments for patients and offer good care.

Access, Cost-effectiveness and Formulary Decisions :
Due to their high cost, pharmacists are involved in the assessment of the affordability and accessibility of gene therapies. They are also involved in hospital formulary decisions to enable the optimal management of gene therapies. [45,46,47,48,49]

Ethical Counselling and Informed Consent Support:
When a patient receives gene therapy, pharmacists can also be a resource for explaining the ethical considerations, advantages, risks, and limitations. Pharmacists can provide the pharmacist's role as part of the decision-making process.

Pharmacist Participation in Clinical Research and Trials :
In clinical trials, for example, pharmacists can ensure the right medication is prepared and stored, dispensed and used in the right way and regularly monitored for safety. [30,31,32,34,35,36]

Research Gaps:

All five so-called gaps are real and documented but the evidence base is uneven. Long-term safety and delivery are directly studied; cost-effectiveness and regulatory standardization are mostly described as unmet needs rather than measured. The matrix below shows the facts of where the coverage of the retrieved literature is. [25,26,27,28,29,47,48,49]

Limited long term safety Data :
Long-Term Safety and Off-Target Identification [25,26,27,28,29]
Most obviously, there is a temporal gap: gene-editing trials are almost all early phase, and follow-up tapers away just where late effects would emerge. In a scoping review of 29 published trials (420 patients), edited cells were detected at 3 and 6 months in most studies, but later timepoints were reported far less frequently, and long-term oncogenic surveillance outcomes have not been addressed.Reviews converge on the same point: continuous post-treatment monitoring is required for late-arising immune responses, off-target edits, and oncogenic risk, and longer-term studies of base and prime editors are missing.
Where they exist, data are reassuring but short-term. Several AAV gene therapy trials have not observed genotoxic effects, and integration-site analyzes in patients and primates did not show enrichment at cancer-k is the bottleneck: comprehensive genotoxicity assessment of programmable nucleases in human hematopoietic stem cells remains challenging with current methods. Off-target activity varies by nuclease platform, donor DNA and target sequence.
Newer work highlights large structural variations — translocations and megabase-scale deletions — as an under-valued risk class

Lack of efficient tissue – Specific delivery
Delivery, Diversity and Evidence of Economic Value [21,22,23,46]

Engineering tissue-specific delivery remains a challenge. Accessing privileged tissues such as the CNS is technically challenging, targeting post-mitotic tissue is complicated by limited regenerative capacity, and AAV vectors have limited transduction, off-target biodistribution, immune recognition, and poor preclinical-to-clinical translatability.
In a one-year study in mice, AAV-CRISPR led to sustained restoration of dystrophin but also revealed unintended changes to the genome and transcript .
Detection is the bottleneck: comprehensive genotoxicity assessment of programmable nucleases in human hematopoietic stem cells remains challenging with current methods . Off-target activity varies by nuclease platform, donor DNA and target sequence .
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Underrepresentation of diverse populations in trials, Limited Proof of cost-effectiveness:
Under representation of diverse populations in trials and 12.4 limited Vectors screened in animals may underperform in human tissue due to species-specific receptor expression . Emerging platforms—exosome-delivered Cas9 RNPs, engineered capsids, LNPs—show targeted accumulation in proof-of-concept models but remain preclinical . On diversity, the deficit is measurable. Among 20,692 US trials, only 43% reported

any race/ethnicity data, median minority enrollment (27.6%) fell below census estimates, and industry funding was associated with lower reporting and lower minority enrollment Turner . This matters mechanistically for gene editing: roughly 8% of individuals . Cost-effectiveness evidence exists but is fragile. Across 26 CEAs of seven FDA-approved cell and gene therapies, about half fell below $100,000–$150,000 per QALY, yet results varied from very cost-effective to far from it for the same treatment, driven by differing long-term extrapolations from short, single-arm trials . Similar instability appears in hemophilia, where modeled value depends on assuming durability of at least 10 years. LMIC-adapted analyses are rare; a Uganda sickle cell framework found could be cost-effective at scaled cost from a societal perspective . What the Gaps Mean The five gaps are not independent. Short trial follow-up starves the economic models of durability data, non-representative genomes and populations weaken off-target prediction, and fragmented real-world data infrastructure—94% of adverse events estimated unreported—undermines post-market surveillance carry a variant creating a distinct off-target site for exa-cel, and the reference genome used for target design has limited demographic representation . Proposed remedies center on community-engaged co-design and organizational commitment, partnerships, accountability, and resources [30,31,32,33,39,40,41,47,48]

Need for standardized off-target detection and regulatory guidelines [25,26,27,28,29,47,48,49]

. Regulatory harmonization of long-term follow-up and standardized off-target assays are repeatedly named as prerequisites rather than solved problems . These are research priorities, not clinical guidance; individual treatment decisions belong with a licensed clinicians.

Future Perspectives:

CRISPR – Cas genome editing:

Has advanced into approved clinical treatments, yet overcoming genotoxicity, structural variations, and delivery constraints remains the central focus for next-generation platforms .Emerging strategies combine novel biochemical architectures, high-resolution surveillance, and programmable delivery vehicles to improve safety profiles. [17,18,19,20,25,26,27]

Next-Generation Precision Editing Modalities

Conventional Cas9 nucleases induce targeted DNA double-strand breaks (DSBs), which frequently trigger complex genomic alterations including chromosomal translocations, large deletions, and chromothripsis .To mitigate these risks, modern development is pivoting toward DSB-independent platforms

. Base editors (Bes) enable targeted single-nucleotide conversions, while prime editors (Pes) utilize reverse transcriptase fusions to write precise insertions, deletions, or substitutions without requiring exogenous donor DNA templates

Beyond sequence alterations, dead-Cas (dCas9) transcriptional platforms like CRISPR interference (CRISPRi) and activation (CRISPRa) allow reversible gene regulation and epigenetic remodeling without altering primary DNA sequences .

Additionally, RNA-targeting Cas13 systems and endogenous ADAR-recruiting tools provide transient transcriptome modulation, eliminating permanent genomic risks entirely.

Multiplex Editing and Combinatorial CRISPR Therapeutics:

CRISPR therapeutics increasingly rely on multiplex editing and combination strategies to address polygenic diseases, engineer advanced cell therapies, and mitigate therapeutic resistance.

Investigating these strategies requires examining the primary clinical applications, the engineering platforms driving multiplex delivery, and the mitigation of genomic safety hazards . [5,6,34,35,36]

Adoptive Cell Therapies [34,35,36]

Multiplex CRISPR editing is widely implemented in ex vivo adoptive cell therapy to generate allogeneic, “off-the-shelf” chimeric antigen receptor (CAR) T cells and combat tumor immunosuppression.Simultaneous disruption of multiple loci disrupts endogenous immune checkpoints and graft-versus-host pathways while introducing therapeutic receptors .

Simultaneous knockout of TRAC and B2M prevents graft-versus-host disease and host-mediated allorejection in allogeneic CAR T cells .

Combinatorial ablation of checkpoint receptors like PDCD1 and CTLA4 overcomes immunosuppressive tumor signals and improves T cell persistence.

Multiplex disruption of apoptotic receptors, including FAS, confers resistance to activation-induced cell death in engineered lymphocytes.

Clinical CRISPR translation:

It is actively expanding from rare monogenic disorders into common and polygenic conditions, though most applications remain in preclinical stages or early human trails.While monogenic conditions offer clear single-gene targets, multifactorial disorders involve intricate networks of risk alleles, epigenetic dysregulation, and non-coding variations that require advanced multiplexing or transcriptional modulation .

Clinical Pipeline and Therapeutic Claims [30,31,32,33,39,40,41]

Targeting common complex diseases requires shifting therapeutic logic from simple DNA double-strand breaks to precise base modification, transcriptional tuning, or cellular engineering .

In vivo disruption of single nodal genes (such as PCSK9 and ANGPTL3) significantly lowers serum lipids in common cardiovascular risk.

Nodal Targets in Common Cardiovascular Disease

Cardiovascular disorders represent the most advanced setting for translating gene editing to polygenic trait. [39,40,41]

.Rather than attempting to correct hundreds of subtle polygenic risk variants simultaneously, therapeutic strategies typically target central rate-limiting pathways

Lipid nanoparticle-mediated base editing designed to inactivate PCSK9 has reached early clinical trials, demonstrating durable reductions in low-density lipoprotein cholesterol.Preclinical pipelines are similarly

pursuing the inactivation of ANGPTL3 and APOB to achieve broader multigene control of dyslipidemia .Structural and electrical cardiomyopathies, precise platforms such as base editing and prime editing are restoring function in MYH7, MYBPC3, and RYR2 variants while avoiding cytotoxic double-strand DNA breaks .

Epigenetic interventions and CRISPR activation tools are also being explored to reprogram non-myocyte cardiac cells into cardiomyocyte-like lineages to mitigate post-infarction scarring .

Metabolic, Neurodegenerative, and Renal Indications:

CRISPR applications are also diversifying across other complex chronic pathologies: [33,38,39,40,41]

In type 1 diabetes, hypoimmunogenic stem cell therapy utilizes ex vivo multi-locus CRISPR editing (B2M, PD-L1, HLA-E) to protect allogeneic islet cells from immune rejection.

For neurodegenerative disorders, preclinical editing converted pathogenic APOE4 alleles into the neuroprotective APOE2 variant to modify Alzheimer's susceptibility.

In polygenic nephropathies, targeted disruption of APOL1 risk alleles provides a viable therapeutic avenue for hypertensive glomerulosclerosis.

Epigenomic editing modulates disease-associated hypomethylation at specific promoter regions, rescuing podocyte function without altering the underlying nucleotide sequence.

Point-of-Care CRISPR Manufacturing: [21,22,23,46]

Deploying scalable, affordable, and point-of-care (POC) manufacturing for CRISPR-based therapeutics requires overcoming high centralized production costs through closed-system automation, low-cost non-viral delivery platforms, and decentralized quality control frameworks This synthesis examines current engineering solutions across closed automated systems, non-viral delivery platforms, and decentralized regulatory frameworks.

Closed-System Automation

Automated, closed-system devices enable the production of gene-edited cellular therapeutics directly in hospital settings without cleanrooms.

Automated clinical devices, such as the CliniMACS Prodigy system, allow closed-system electroporation of up to 100 million hematopoietic stem cells targeting BCL11A with high editing efficiency .

Modular isolator-based platforms facilitate on-demand therapeutic generation near clinical bedsides, which minimizes vein-to-vein transit intervals and preserves cell viability .

Tabletop automated microfluidic instruments consolidate liquid handling steps into compact point-of-care systems, avoiding open-handling contamination .

Non-Viral Delivery Platforms

Transitioning from viral vectors to synthetic or biological delivery methods dramatically lowers upfront consumable costs and accelerates point-of-care processing

Reagent shelf-life remains an essential variable for distributed manufacturing hubs. Formulations using lyophilized RNP complexes circumvent strict ultralow-temperature cold-chain bottlenecks, simplifying on-site reconstitution in resource-constrained environments.

Integration with AI, omics, and synthetic biology for CRISPR-based therapeutics:

Artificial intelligence, multi-omics, and synthetic biology for CRISPR genome editing therapeutics. [20,24,25,26]

Machine learning, genomics, transcriptomics, proteomics, and synthetic biology in CRISPR therapeutic development.

AI, Omics, and Synthetic Biology in CRISPR Therapeutics

The clinical translation of CRISPR-based therapeutics increasingly relies on three complementary pillars: computational models from artificial intelligence (AI), high-resolution biological datasets from multi-omics platforms, and rational circuit design from synthetic biology .

Artificial Intelligence in Precision Editing

Machine learning and deep learning architectures streamline guide RNA (gRNA) selection, off-target liability prediction, and enzyme engineering to ensure safety and therapeutic efficacy.Algorithms mitigate laborious empirical testing by calculating DNA repair profiles and cleavage activity in silico before clinical deployment . [20,24,25,26]

Predictive platforms like DeepCRISPR, DeepHF, and CRISPOR optimize gRNA selection by modeling sequence features, chromatin context, and off-target liabilities Generative diffusion architectures and protein language models design de novo nucleases and engineered Cas effectors with expanded targeting space and minimized genotoxicity

Deep learning models accurately forecast repair outcomes—such as insertion-deletion patterns—by evaluating cellular states beyond isolated sequence matching.

Multi-Omics Profiling and Target Discovery [10,20,24,25,26]

Comprehensive multi-omics layers provide the physiological context required to characterize disease targets, uncover resistance mechanisms, and evaluate off-target genotoxicity .Integrating genomics, single-cell transcriptomics, and epigenomics reveals functional cellular heterogeneity that single-modality approaches .

Multiplexed functional screens such as Perturb-seq and CROP-seq directly link pooled CRISPR perturbations to single-cell transcriptomic readouts.

Epigenomic mapping tools like Perturb-ATAC and Spear-ATAC assess chromatin accessibility and histone modifications to reveal chromatin-dependent editing .

Integrated patient omics profiles accelerate biomarker identification, patient stratification for clinical trials, and personalized gRNA design for heterogeneous diseases

Synthetic Biology and Next-Generation Therapeutic Systems [5,6,10,20]

Synthetic biology establishes systematic design-build-test-learn frameworks to assemble multi-component effectors, modular delivery vehicles, and dynamic therapeutic circuits .

These tools enable complex genetic logic, viral countermeasure deployment, and safer in vivo gene correction.

Global Access and Policy Frameworks for CRISPR Therapeutics: [45,46,47,48,49,50]

The clinical translation of CRISPR-Cas9 technologies has reached fruition with regulatory approvals for hemoglobinopathies, yet global implementation requires resolving severe disparities in economic access, regulatory convergence, and local healthcare infrastructure.

Establishing an actionable framework demands examining three core facets: therapeutic pricing models, international regulatory harmonization, and infrastructure building across resource-limited settings.

Pricing Models and Economic Sustainability

Initial approvals of commercial CRISPR therapies, such as exagamglogene autotemcel carry list prices between $2.2 million and several million dollars per patient Because development relies on public-private mechanisms originating in academia before commercial licensing, alternative distribution models are necessary to prevent these therapies from remaining an exclusive privilege.

Academic licensing conditions and non-profit development models can reduce per-patient costs tenfold compared to conventional commercial pricing structures. Implementing outcome-linked payments and risk-sharing agreements distributes financial risk across public healthcare programs and pharmaceutical developers.

Integrating multidimensional metrics, such as the Genomic Equity Accessibility Framework, helps quantify distributive equity alongside direct manufacturing costs

Infrastructure and Delivery in Low- and Middle-Income Countries

The geographical burden of diseases targeted by early CRISPR interventions resides predominantly in resource-limited regions, particularly sickle cell disease.

Despite clinical efficacy, current ex vivo paradigms require intensive cell handling, specialized apheresis centers, and bone marrow transplantation facilities that are largely absent in high-burden countries.

CONCLUSION:

Future Horizons [4,7,10,20,30,31,32,33,34,35,36]

CRISPR-Cas systems have transitioned from prokaryotic immune mechanisms into dominant platforms for targeted genome editing across medicine, agriculture, and biotechnology .

While therapeutic and agricultural translation has advanced rapidly, overcoming clinical delivery hurdles, unintended genetic disruptions, and regulatory boundaries remains essential for standard adoption.

Transformative Scope Across Disciplines [4,7,30,31,32,33,34,35,36]

CRISPR-Cas technology has demonstrated marked advantages over preceding platforms like zinc-finger nucleases and TALENs due to its multiplexing capacity, ease of design, and low development cost, In biomedical research, the system accelerates disease modeling, enables high-throughput pooled genetic screening, and clarifies complex signaling cascades.

Early clinical interventions have confirmed therapeutic feasibility in human patients for genetic blood disorders such as sickle cell disease and β-thalassemia, as well as emerging oncology and infectious disease paradigms .

DECLARATIONS

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review article.

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Author Contributions

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Tables and Figures

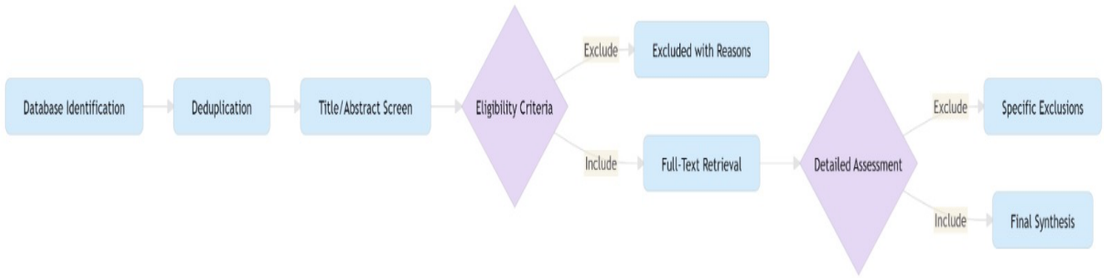

Figure 1 – CRISPR genome-editing workflow

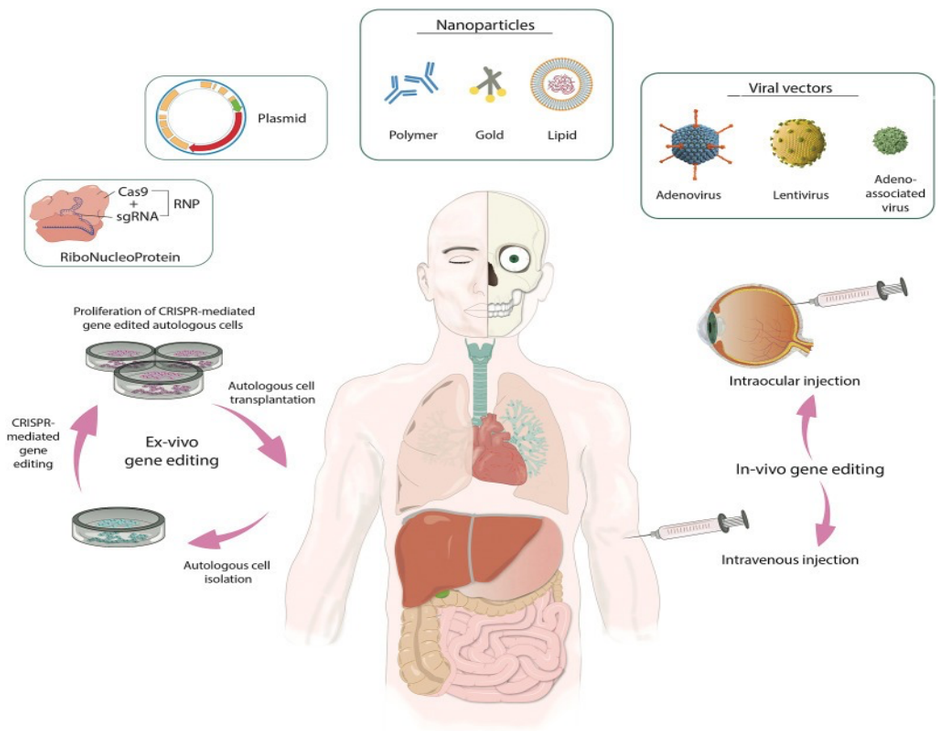

Figure 1. Examples of CRISPR delivery systems and ex vivo versus in vivo editing.

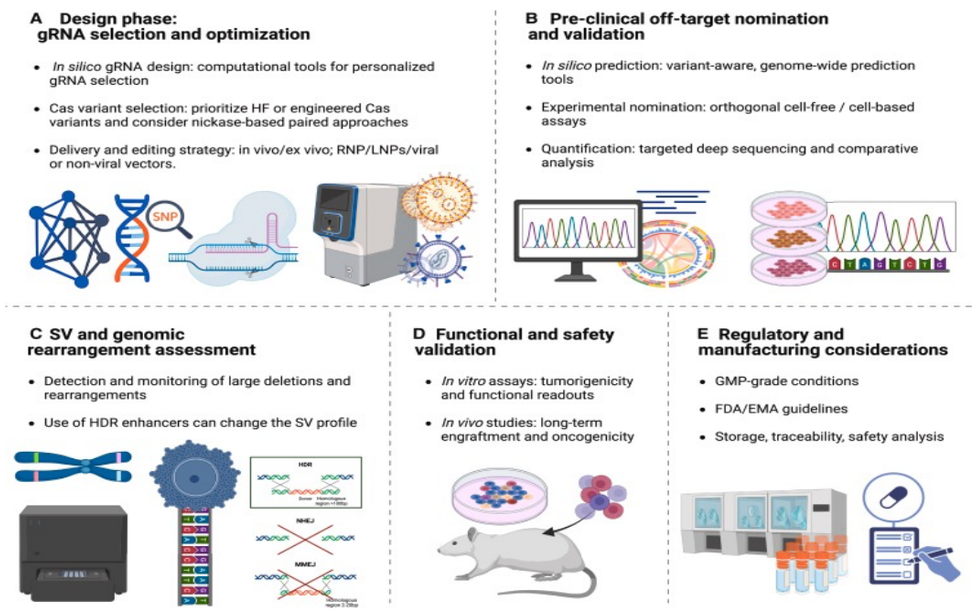

Figure 2. On-target and off-target recognition, genomic outcomes and alternative editing systems.

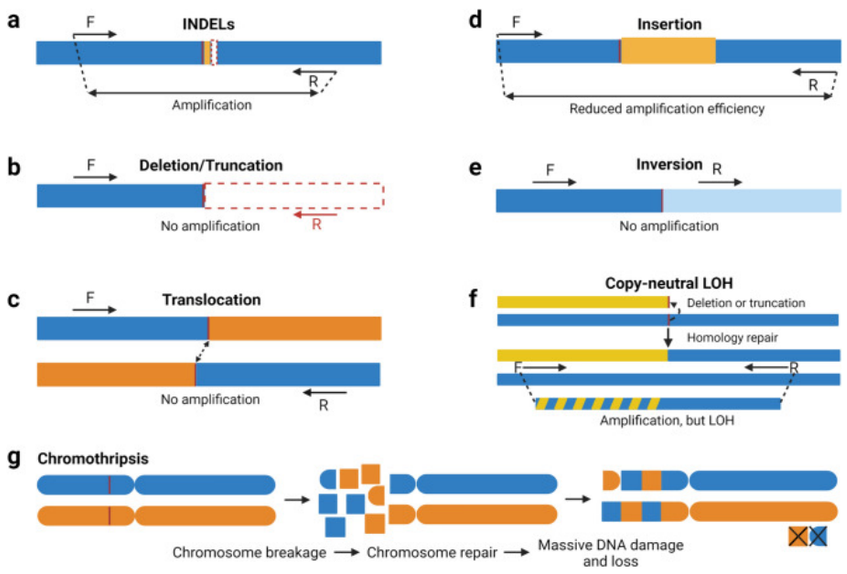

Figure 3. Workflow for guide design, off-target validation, structural-variant assessment and safety testing

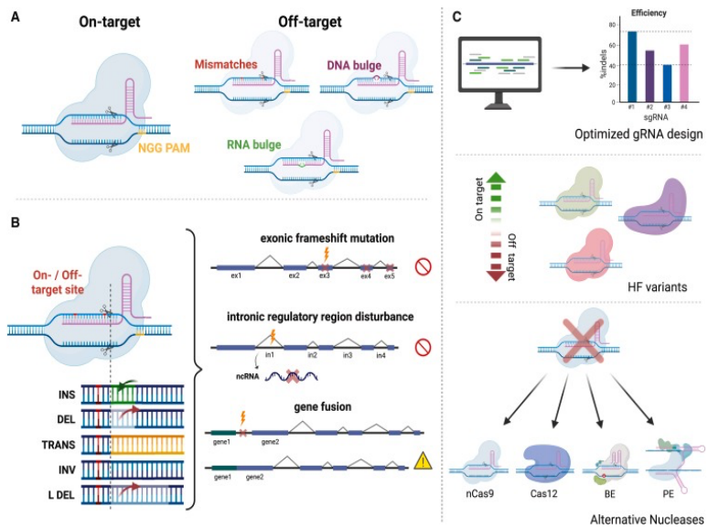

Figure 4. Examples of structural variants and chromosome damage that require assessment.

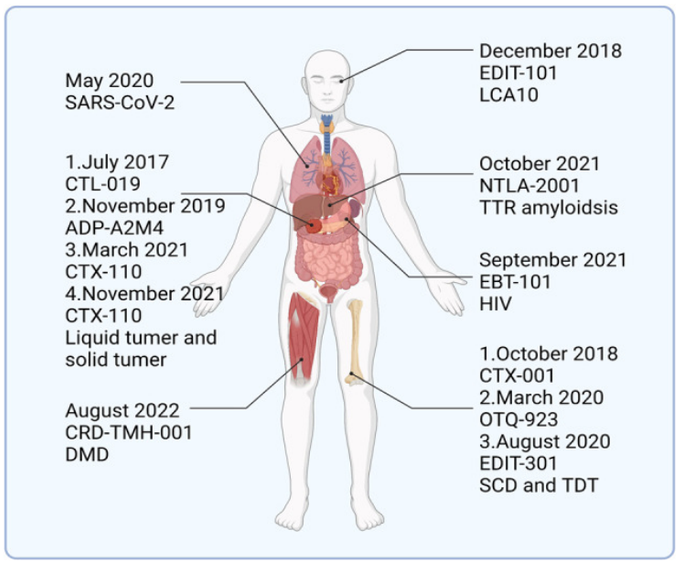

Figure 5. Selected examples of clinical and investigational CRISPR programmes across organs and diseases.

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