https://doi.org/10.62587/BMPR.2.2.2026.50-65 ISSN: 3136-5248

Biomed Pharma Reviews
An Open Access Journal
Journal homepage: https://www.biomedpharmareviews.com

Review Article **Open Access**

Recent advances in CRISPR-Cas systems and their applications in biological research

Florento Eric Zkayi[1*]

[1]Faculty of Human Medicine, Simon Kimbangu Univeristy, Commune Kalamu, Kinshasa, Democratic Republic of the Congo. E-mail: Zkayi.eric@hotmail.com

Article Info

Volume 2, Issue 2, July 2026
Received : 11 January 2026
Accepted : 10 May 2026
Published : 25 July 2026
doi: 10.62587/BMPR.2.2.2026.50-65

Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated (Cas) proteins have transformed from an obscure prokaryotic adaptive immune mechanism into the most versatile toolkit in modern molecular biology. Within little more than a decade of the demonstration that Cas9 could be programmed to cleave defined genomic sequences, the field has progressed to the first regulatory approval of a CRISPR-based medicine and to bespoke, patient-specific therapies delivered directly in the body. This narrative review synthesizes developments from 2019 onward, situated against foundational earlier work, to provide an integrated and critical account of where the technology now stands. We first revisit the biological logic of CRISPR immunity and the structural basis of RNA-guided DNA recognition, because these principles continue to constrain and inspire engineering. We then examine the maturation of precision editing modalities-high-fidelity nucleases, cytosine and adenine base editors, and prime editors-analyzing the mechanistic trade-offs that govern their efficiency, product purity, and off-target behavior. Parallel advances in transcriptional and epigenetic modulation, RNA-targeting effectors, and CRISPR-based diagnostics are appraised for their distinct value propositions. A recurring theme is the tension between editing potency and specificity, and between the breadth of correctable mutations and the practicality of delivery. We compare conflicting reports on unintended consequences, including large structural rearrangements, activation of the p53 pathway, and editor-dependent off-target mutagenesis, and we weigh their translational implications. Landmark clinical results in haemoglobinopathies, transthyretin amyloidosis, and ultra-rare metabolic disease are evaluated critically, alongside persistent barriers of extrahepatic delivery, immunogenicity, equitable access, and germline governance. We conclude by identifying priority research gaps and plausible near-term directions, arguing that the decisive challenges are now as much translational and ethical as they are molecular.

Keywords: *CRISPR-Cas9, Base editing, Prime editing, Genome editing, Gene therapy, Cas13, Off-target effects, Therapeutic delivery*

1. Introduction

1.1. Background

The recognition that prokaryotes possess a heritable, sequence-specific defence against invading nucleic

* *Corresponding author: Florento Eric Zkayi, Faculty of Human Medicine, Simon Kimbangu Univeristy, Commune Kalamu, Kinshasa, Democratic Republic of the Congo. E-mail: Zkayi.eric@hotmail.com*

acids reframed a set of repetitive genomic loci that had puzzled microbiologists for two decades. Early sequence surveys noted regularly spaced repeats interrupted by unique intervening sequences, and comparative analyses later revealed that many of these spacers matched bacteriophage and plasmid sequences, hinting at a memory of prior infection (Ishino *et al.*, 1987; Mojica *et al.*, 2005; Bolotin *et al.*, 2005). The functional proof that such loci confer acquired resistance to phage established CRISPR as a bona fide adaptive immune system (Barrangou *et al.*, 2007). Subsequent work dissected the three-phase logic of adaptation, expression, and interference, showing that small CRISPR RNAs guide effector complexes to complementary targets and that, in type II systems, the nuclease acts on DNA rather than RNA (Brouns *et al.*, 2008; Marraffini and Sontheimer, 2008; Garneau *et al.*, 2010). The identification of a trans-activating RNA required for crRNA maturation, and the reconstitution of a dual-RNA-guided Cas9 ribonucleoprotein capable of programmable double-strand cleavage, completed the conceptual bridge from immunity to engineering (Deltcheva *et al.*, 2011; Gasiunas *et al.*, 2012; Jinek *et al.*, 2012).

1.2. Importance of the topic

What distinguishes CRISPR from earlier targetable nucleases is the substitution of protein–DNA recognition with Watson–Crick base pairing: retargeting requires only a change of guide sequence rather than laborious protein re-engineering. This single property democratised genome manipulation, and its rapid adaptation to mammalian cells catalysed applications across basic discovery, disease modelling, agriculture, and therapeutics (Cong *et al.*, 2013; Mali *et al.*, 2013; Doudna and Charpentier, 2014). The technology has since diversified into a family of tools whose common thread is programmable, RNA-guided targeting of nucleic acids, extending far beyond the original double-strand break paradigm (Knott and Doudna, 2018; Pickar-Oliver and Gersbach, 2019; Anzalone *et al.*, 2020).

1.3. Global relevance

The reach of CRISPR is genuinely global. In research it has become a default method for interrogating gene function; in medicine it underpins therapies for diseases that disproportionately affect specific populations, such as sickle cell disease, which is most prevalent in sub-Saharan Africa, the Middle East, and South Asia (Frangoul *et al.*, 2021). In food security, editing accelerates the introduction of agronomic traits without the regulatory and public-acceptance burdens associated with transgenesis in many jurisdictions (Chen *et al.*, 2019). During the COVID-19 pandemic, CRISPR-based assays illustrated the platform's adaptability to point-of-need diagnostics (Broughton *et al.*, 2020). This breadth also raises questions of equitable access and governance that are inherently international in scope (Lander *et al.*, 2019).

1.4. Current challenges

Despite remarkable momentum, several problems remain incompletely solved. Unintended editing outcomes—ranging from small insertions and deletions at off-target sites to large deletions and chromosomal rearrangements at the on-target locus—complicate safety assessment (Kosicki *et al.*, 2018). Cellular responses to double-strand breaks, including p53 activation, can bias which cells survive editing (Haapaniemi *et al.*, 2018; Ihry *et al.*, 2018). Pre-existing humoral and cellular immunity to commonly used Cas orthologues may limit in vivo efficacy and raise safety concerns (Charlesworth *et al.*, 2019). Above all, delivering editing machinery efficiently and selectively to the relevant tissue remains the principal bottleneck for most in vivo indications (Doudna, 2020).

1.5. Aim and objectives

This review aims to provide a critical, integrative synthesis of CRISPR-Cas advances with emphasis on the period from 2019 to the present, while anchoring the discussion in foundational studies. Our objectives are: (i) to explain the biological and structural mechanisms underlying contemporary tools; (ii) to compare precision-editing modalities and their trade-offs; (iii) to appraise applications in functional genomics, diagnostics, agriculture, and therapeutics; (iv) to evaluate conflicting evidence on unintended effects; and (v) to delineate research gaps and future directions relevant to clinicians, researchers, and postgraduate audiences.

2. Literature search methodology

This is a narrative rather than a systematic review; nevertheless, a structured and reproducible search strategy

was applied to minimise selection bias. We interrogated PubMed/MEDLINE, Scopus, Web of Science, Embase, and Google Scholar for records published between January 2012 and the date of writing, with particular attention to 2019 onward. Search terms combined controlled vocabulary and free-text keywords, including *CRISPR*, *Cas9*, *Cas12*, *Cas13*, *base editing*, *prime editing*, *genome editing*, *gene therapy*, *off-target*, and *delivery*, linked with Boolean operators and truncation.

Inclusion criteria were peer-reviewed original research and authoritative reviews written in English that reported mechanistic, methodological, or translational advances in CRISPR-Cas systems; landmark pre-2019 studies were retained where they were foundational to the narrative. Exclusion criteria were non-peer-reviewed preprints used as primary evidence, conference abstracts without full reports, and studies whose claims could not be corroborated by the published record. Reference lists of key reviews were hand-searched to identify additional sources (snowballing). Given the narrative design, no formal meta-analytic pooling or quantitative risk-of-bias scoring was performed, and the selection reflects a degree of expert judgement, which we acknowledge as a limitation. A schematic of the screening process is provided in Figure 1.

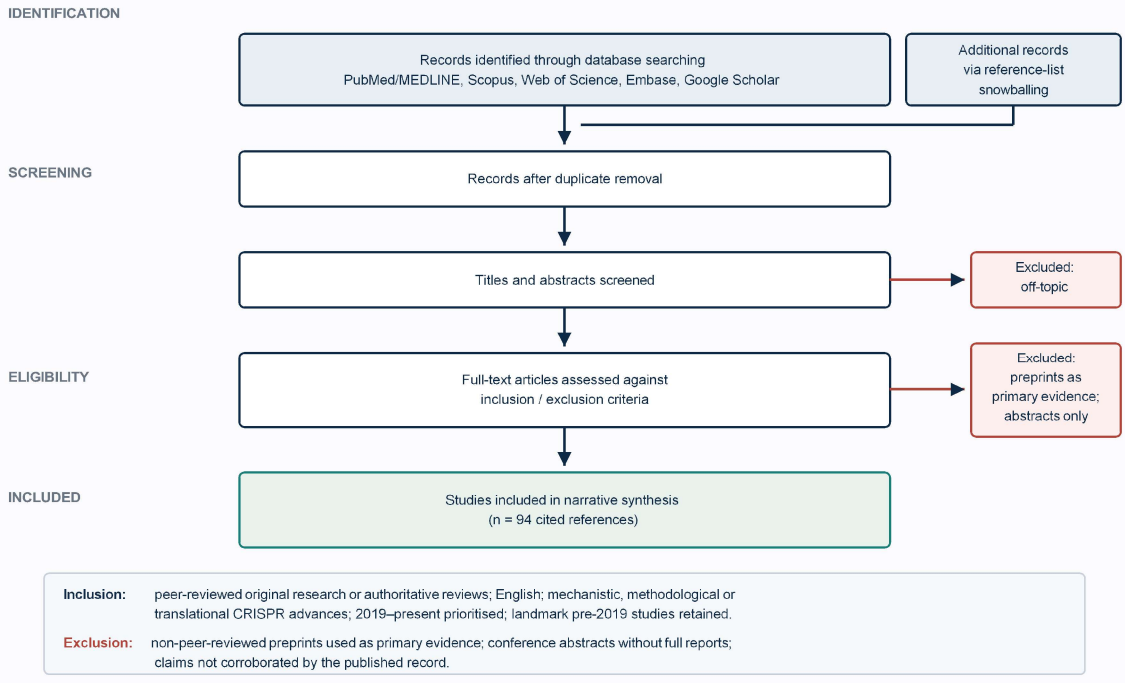

Figure 1: Flow diagram of the literature search and selection process. Records were identified across five databases and supplemented by reference-list snowballing, then screened at title/abstract and full-text level against the stated inclusion and exclusion criteria. Because this is a narrative rather than a systematic review, stage-wise record counts were not tracked prospectively; the diagram depicts the selection logic, with the final synthesised corpus corresponding to the 94 cited references

3. Biological foundations and the CRISPR-Cas toolkit

3.1. Architecture and diversity of CRISPR-Cas systems

CRISPR-Cas systems are classified into two broad classes and multiple types on the basis of their effector modules. Class 1 systems (types I, III, and IV) employ multi-subunit effector complexes, whereas class 2 systems (types II, V, and VI) rely on a single large multidomain protein—Cas9, Cas12, and Cas13, respectively (Makarova *et al.*, 2020). This taxonomy is not merely descriptive: the single-protein architecture of class 2 effectors is precisely what makes them tractable for heterologous deployment, explaining why they dominate the engineering literature. Evolutionary reconstructions suggest that these effectors arose repeatedly from mobile genetic elements, a history that continues to be mined for novel enzymes with useful properties such as compact size or distinct Protospacer-Adjacent Motif (PAM) requirements (Koonin and Makarova, 2019). Understanding this diversity matters because each type imposes characteristic constraints—target sequence, PAM, product (DNA versus RNA)—that determine its suitability for a given task.

3.2. Mechanism of RNA-guided DNA recognition and cleavage by Cas9

The archetypal type II effector, *Streptococcus pyogenes* Cas9 (SpCas9), recognises a 52 -NGG-32 PAM and, when loaded with a single guide RNA (sgRNA), interrogates the genome for complementary protospacers. Single-molecule and structural studies established that PAM recognition licenses local DNA unwinding, followed by directional RNA–DNA heteroduplex (R-loop) formation that, if complete, positions the HNH and RuvC nuclease domains to cleave the target and non-target strands, generating a blunt double-strand break (Sternberg *et al.*, 2014; Nishimasu *et al.*, 2014; Jiang and Doudna, 2017). Critically, seed-region complementarity proximal to the PAM dominates target discrimination, which both explains characteristic off-target patterns and provides a rational basis for specificity engineering. The engineering of the crRNA:tracrRNA duplex into a chimeric sgRNA simplified the system to two components and remains the practical foundation of most applications (Ran *et al.*, 2013). These steps are depicted in Figure 2.

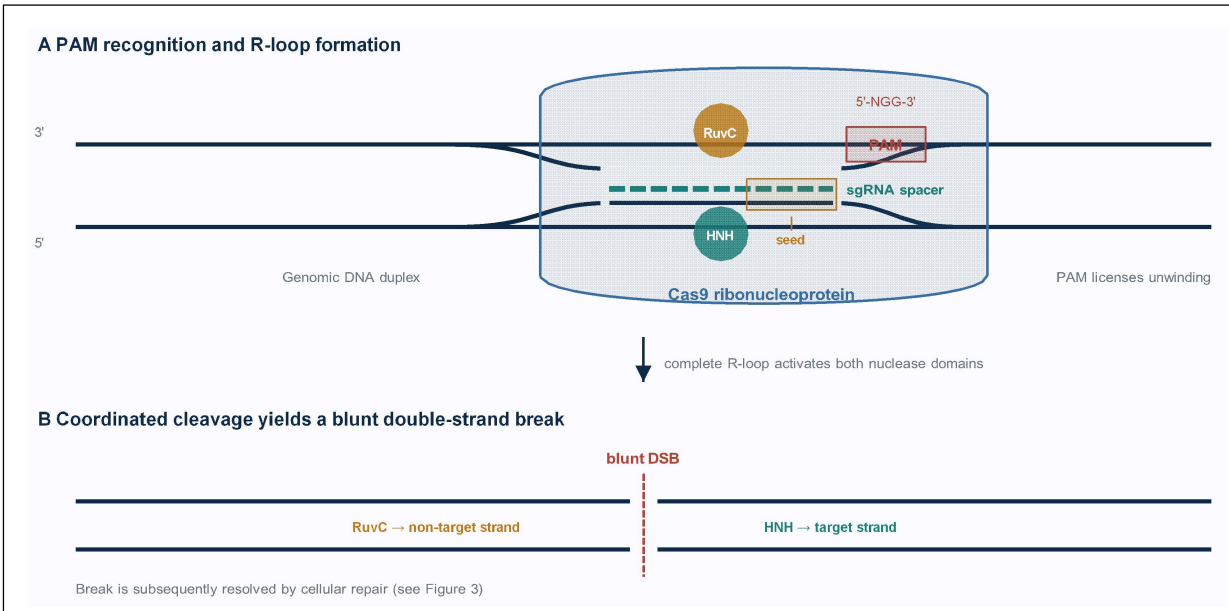

Figure 2: Mechanism of RNA-guided DNA recognition and cleavage by Cas9. (A) The Cas9–sgRNA ribonucleoprotein interrogates DNA for a Protospacer-Adjacent Motif (PAM). PAM binding licenses local duplex unwinding and directional R-loop formation, in which the sgRNA spacer base-pairs with the target strand. The PAM-proximal seed region is the dominant determinant of target discrimination and therefore of off-target behaviour. (B) A complete R-loop allosterically activates both nuclease domains: HNH cleaves the target strand and RuvC the non-target strand, generating a blunt double-strand break that is subsequently resolved by cellular repair

3.3. DNA repair outcomes and their exploitation

The biological consequence of a Cas9-induced break depends on which repair pathway resolves it. Error-prone Non-Homologous End Joining (NHEJ) predominates in most cell types and typically produces small insertions or deletions that can disrupt a coding sequence—ideal for gene knockout. Homology-Directed Repair (HDR), which uses a supplied template to install precise edits, is comparatively inefficient and largely restricted to dividing cells, a limitation that has motivated the development of template-free precision tools. Strategies to shift the balance toward precise outcomes, or to reduce the burden of double-strand breaks altogether, recur throughout the subsequent evolution of the field and directly motivated base and prime editing.

3.4. Engineering specificity: High-fidelity nucleases and off-target detection

Early reports that CRISPR nucleases could cleave off-target sites at appreciable frequencies tempered initial enthusiasm and set a research agenda that persists today (Hsu *et al.*, 2013; Fu *et al.*, 2013). Two complementary responses emerged. The first was empirical off-target discovery: unbiased genome-wide methods such as GUIDE-seq and CIRCLE-seq allowed off-target sites to be catalogued rather than merely predicted, revealing that computational prediction alone is insufficient (Tsai *et al.*, 2015; Tsai *et al.*, 2017). The second was protein

engineering. Truncated guides, paired nickases, and rationally or evolutionarily engineered high-fidelity variants (eSpCas9, SpCas9-HF1, HypaCas9, evoCas9) substantially reduced off-target activity, in several cases to undetectable levels by contemporary assays (Fu *et al.*, 2014; Ran *et al.*, 2013; Slaymaker *et al.*, 2016; Kleinstiver *et al.*, 2016; Chen *et al.*, 2017; Casini *et al.*, 2018). In parallel, PAM-relaxed variants (xCas9, SpG, SpRY) widened the addressable genome, trading some specificity for expanded targeting range (Hu *et al.*, 2018; Walton *et al.*, 2020; Kleinstiver *et al.*, 2015). A candid reading of this literature is that fidelity and flexibility are frequently in tension, and that "no detectable off-targets" is an assay-dependent claim rather than an absolute one.

3.5. Cellular responses and structural consequences of editing

Two lines of evidence complicated the assumption that on-target editing is inherently benign. First, independent groups reported that Cas9-induced double-strand breaks activate a p53-mediated damage response, such that cells tolerating editing may be enriched for compromised p53 function—a theoretical oncogenic concern, particularly for ex vivo expanded products. Second, careful long-read and clonal analyses showed that repair of on-target breaks can generate large deletions and complex rearrangements that short-amplicon sequencing misses, meaning that standard indel quantification may underestimate genotoxic risk. These findings are not universally reproduced across cell types and contexts, and their clinical significance remains debated; nonetheless, they justify the shift toward break-free editing chemistries and toward more comprehensive genotoxicity screening in therapeutic development.

Table 1: Summary of selected landmark studies in the development of CRISPR-Cas technologies

Study / contribution	Key advance	Significance
Barrangou *et al.* (2007)	CRISPR confers phage resistance	Established CRISPR as an adaptive immune system
Jinek *et al.* (2012)	Programmable dual-RNA-guided Cas9 cleavage	Conceptual basis for CRISPR genome editing
Cong *et al.* (2013) and Mali *et al.* (2013)	Editing in mammalian cells	Enabled broad biological application
Komor *et al.* (2016)	Cytosine base editing	Precise single-base change without DSB
Gaudelli *et al.* (2017)	Adenine base editing	Expanded correctable transition mutations
Anzalone *et al.* (2019)	Prime editing	Versatile, template-free precise editing
Frangoul *et al.* (2021)	Ex vivo editing for SCD/β-thalassaemia	Clinical proof of curative potential
Gillmore *et al.* (2021)	*In vivo TTR* editing (systemic)	First in-body CRISPR editing in humans
Musunuru *et al.* (2025)	Patient-specific in vivo base editing	First bespoke n-of-1 CRISPR therapy

4. Precision editing beyond the double-strand break

4.1. Base editing

Base editors couple a catalytically impaired Cas9 (a nickase or dead enzyme) to a nucleotide-modifying enzyme, enabling direct chemical conversion of one base to another without a double-strand break or donor template. Cytosine Base Editors (CBEs) fuse a cytidine deaminase to install C•G-to-T•A transitions, while Adenine Base Editors (ABEs) employ a laboratory-evolved deoxyadenosine deaminase to achieve A•T-to-G•C conversions—together addressing a substantial fraction of known pathogenic point mutations (Komor *et al.*, 2016; Gaudelli *et al.*, 2017; Rees and Liu, 2018). Iterative engineering improved editor expression, narrowed the activity window, and enhanced product purity (Koblan *et al.*, 2018). However, base editing is not free of unintended activity: CBEs, in particular, were shown to generate Cas9-independent, genome-wide single-nucleotide variants in mouse embryos and in rice, and both editor classes can cause transcriptome-wide off-target RNA edits, effects that were subsequently mitigated by deaminase engineering (Zuo *et al.*, 2019; Jin *et al.*, 2019; Grünewald *et al.*, 2019). The therapeutic promise is nonetheless concrete: base editing corrected the sickle mutation in human haematopoietic stem cells and durably lowered PCSK9 and LDL cholesterol after a single in vivo dose in non-human primates, establishing proof of concept for systemic base editing (Newby *et al.*, 2021; Musunuru *et al.*, 2021; Rothgangl *et al.*, 2021).

4.2. Prime editing

Prime editing extended precision further by writing new genetic information directly into a target site. The system pairs a Cas9 nickase fused to an engineered reverse transcriptase with a prime editing guide RNA (pegRNA) that both specifies the target and templates the desired edit, enabling all transition and transversion point mutations as well as small insertions and deletions without double-strand breaks or donor DNA (Anzalone *et al.*, 2019). Because it does not depend on HDR, prime editing functions in non-dividing cells, a decisive advantage for many therapeutic targets. Early efficiency limitations were addressed by manipulating cellular mismatch repair (PE4/PE5 systems) and by stabilising pegRNAs against degradation (engineered pegRNAs) (Chen *et al.*, 2021; Nelson *et al.*, 2022). Twin prime editing subsequently enabled programmable deletion, replacement, integration, and inversion of larger DNA segments, moving prime editing from point

Table 2: Comparison of major genome-editing modalities

Modality	Molecular action	Edits supported	DSB required	Principal limitation
Nuclease (Cas9/Cas12a)	Targeted double-strand break; NHEJ/HDR	Knockout; HDR insertions	Yes	Indels, large rearrangements, HDR inefficiency (Kosicki *et al.*, 2018)
Cytosine base editor	C→U deamination at nicked site	C•G→T•A	No	DNA/RNA off-target editing (Zuo *et al.*, 2019; Grünewald *et al.*, 2019)
Adenine base editor	A→I deamination at nicked site	A•T→G•C	No	Limited to transitions (Gaudelli *et al.*, 2017)
Prime editor	Nickase + reverse transcriptase (pegRNA)	All substitutions; small indels	No	Lower efficiency; large cargo/delivery (Anzalone *et al.*, 2019)
dCas9 CRISPRi/a	Programmable binding + effector	Reversible expression change	No	Not permanent; off-target binding (Qi *et al.*, 2013)
Cas13 (RNA)	RNA cleavage/RNA base editing	Transient knockdown; A→I(G) RNA edit	No	Transient; collateral activity (Cox *et al.*, 2017)

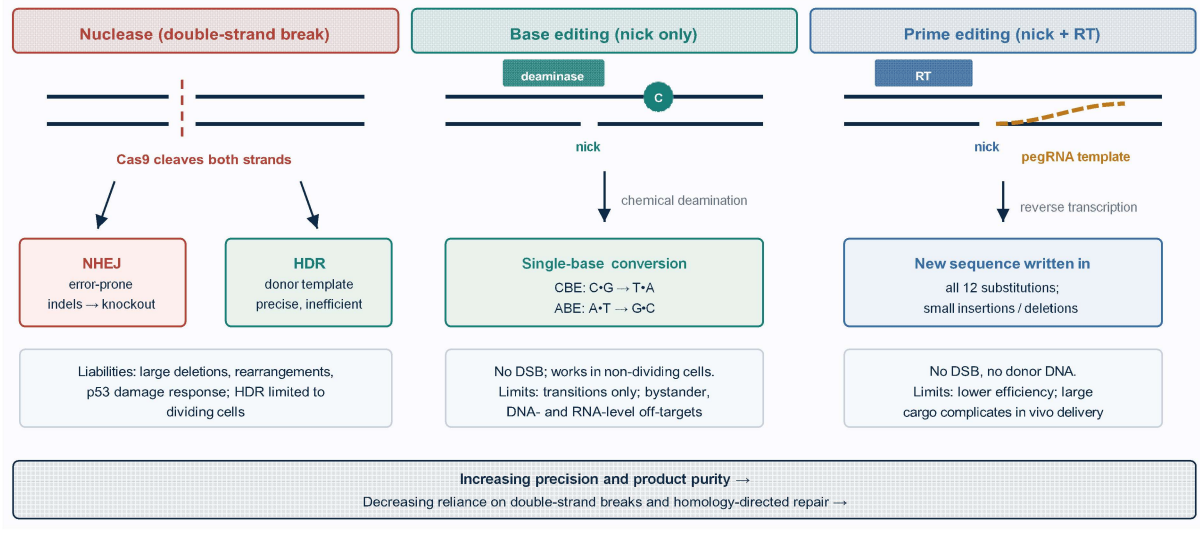

Figure 3: Comparative pathways of the three principal DNA-editing modalities. A nuclease-induced double-strand break is resolved either by error-prone non-homologous end joining, producing indels suited to gene knockout, or by homology-directed repair, which installs precise edits but is inefficient and largely restricted to dividing cells. Base editors act at a nicked site, using a tethered deaminase to chemically convert a single base without a double-strand break. Prime editors couple a nickase to a reverse transcriptase that writes new sequence specified by a pegRNA. The progression from left to right reflects increasing precision and decreasing reliance on double-strand breaks, offset by falling efficiency and rising delivery burden

correction toward genome-scale rewriting(Anzalone *et al.*, 2022). Proof-of-principle correction in patient-derived organoids further supported translational potential, although in vivo delivery of these comparatively large machineries remains a substantial hurdle(Schene *et al.*, 2020; Kantor *et al.*, 2020).

The three DNA-editing modalities are best viewed as complementary rather than competing. Nucleases remain unmatched for efficient knockout and for large insertions via HDR; base editors offer high efficiency for the specific transitions they support; prime editors provide the greatest versatility at the cost, at present, of lower efficiency and greater delivery challenge. Table 2 and Figure 3 summarise these mechanistic distinctions and trade-offs.

5. Regulating and reading the genome without cutting

5.1. Transcriptional and epigenetic modulation

Catalytically dead Cas9 (dCas9) retains programmable DNA binding but not cleavage, making it a modular platform for perturbing gene expression. Fusion or recruitment of repressive or activating effectors yields CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), which tune transcription reversibly and are readily multiplexed (Qi *et al.*, 2013; Gilbert *et al.*, 2013). Potent activators such as the synergistic activation mediator (SAM) complex and VPR expanded the dynamic range of gene induction (Konermann *et al.*, 2015; Chavez *et al.*, 2015). Extending the concept to chromatin, dCas9 fused to catalytic epigenetic writers—for example a p300 acetyltransferase domain—demonstrated targeted, potentially heritable modulation of enhancer and promoter activity without altering the underlying sequence (Hilton *et al.*, 2015). These approaches are valuable precisely because they are non-mutagenic and reversible, an attractive safety profile for applications where permanent sequence change is undesirable, though durability and specificity of epigenetic edits require further scrutiny.

5.2. RNA-targeting effectors and transcriptome engineering

Type VI effectors (Cas13) target RNA rather than DNA, opening a route to transcriptome perturbation that avoids permanent genomic change (Abudayyeh *et al.*, 2016; Abudayyeh *et al.*, 2017). Catalytically inactive Cas13 fused to an adenosine deaminase (the REPAIR/RESCUE systems) enabled programmable RNA base editing, while compact orthologues such as CasRx achieved efficient, specific knockdown of endogenous transcripts, offering an alternative to RNA interference with distinct specificity characteristics (Cox *et al.*, 2017; Konermann *et al.*, 2018). The transient nature of RNA editing is a double-edged feature: it limits durability for chronic indications but reduces the risk of permanent off-target consequences.

5.3. CRISPR-based nucleic acid diagnostics

A serendipitous property of certain class 2 effectors—collateral, indiscriminate nuclease activity triggered upon target recognition—became the basis of a new diagnostic paradigm. Cas13-based SHERLOCK and Cas12a-based DETECTR couple isothermal pre-amplification with target-activated collateral cleavage of reporter molecules to achieve attomolar, single-nucleotide-specific detection (Gootenberg *et al.*, 2017; Chen *et al.*, 2018; Gootenberg *et al.*, 2018). Standardised protocols and multiplexing broadened their utility (Kellner *et al.*, 2019). The clearest demonstration of real-world value came during the COVID-19 pandemic, when Cas12-based assays were rapidly configured for SARS-CoV-2 detection with a workflow suited to decentralised

Table 3: Advantages versus limitations of current CRISPR approaches

Dimension	Advantages	Limitations
Programmability	Retargeting by guide sequence alone	Constrained by PAM availability
Precision editing	Base/prime editing avoid DSBs	Editor-specific off-targets; delivery of large editors
Specificity	High-fidelity variants; unbiased off-target assays	Fidelity–flexibility trade-off; assay-dependent claims
Delivery	LNP and RNP options; hepatic tropism	Extrahepatic delivery unsolved
Clinical	Durable, potentially curative effects	Cost, immunogenicity, irreversibility, equity

testing. These platforms illustrate that CRISPR's impact extends well beyond editing, though competition with mature nucleic acid amplification methods means their niche is defined by portability and instrument-light operation rather than raw sensitivity alone.

6. Applications in functional genomics, agriculture, and disease modelling

Genome-scale CRISPR screens transformed functional genomics by enabling systematic, pooled loss-of-function and gain-of-function interrogation of the genome, accelerating target identification in cancer and elsewhere (Shalem *et al.*, 2014; Wang *et al.*, 2014). The reliability of such screens depends heavily on guide design, and empirically optimised rules markedly improved on-target activity while limiting off-target effects (Doench *et al.*, 2016). Conditional and tissue-specific in vivo editing, exemplified by Cas9-knockin mouse models, made it possible to model tumour genetics with unprecedented flexibility (Platt *et al.*, 2014). In agriculture, CRISPR has been deployed to improve yield, disease resistance, and nutritional traits, often producing edits indistinguishable from those arising through conventional breeding and therefore subject to lighter regulatory oversight in several jurisdictions—an advantage that is as much regulatory as it is technical (Zhang *et al.*, 2018; Gao, 2021). Collectively, these applications underscore that much of CRISPR's scientific value lies in discovery and crop improvement, domains that attract less public attention than therapeutics but arguably deliver broad, near-term benefit.

Table 4: Representative CRISPR-based therapies and technologies with clinical or near-clinical evidence

Agent/platform	Target/indication	Approach	Status/evidence
Exagamglogene autotemcel (exa-cel) (Frangoul *et al.*, 2024)	*BCL11A* enhancer; SCD/β-thalassaemia	Ex vivo Cas9 editing of HSCs	Regulatory approval; durable VOC-free outcomes
NTLA-2001/nexiguran ziclumeran (Fontana *et al.*, 2024)	*TTR*; ATTR amyloidosis/ cardiomyopathy	In vivo LNP Cas9 knockout	Deep, durable TTR reduction in trials
Base editing of PCSK9 (Musunuru *et al.*, 2021)	*PCSK9*; hypercholesterolaemia	In vivo adenine base editing	Durable LDL lowering in primates; clinical development
Bespoke base editor (Musunuru *et al.*, 2025)	*CPS1*; urea-cycle disorder	Individualised in vivo base editing	First n-of-1 patient treated safely
SHERLOCK/DETECTR (Gootenberg *et al.*, 2017; Chen *et al.*, 2018; Broughton *et al.*, 2020)	Nucleic acid detection (incl. SARS-CoV-2)	Cas13/Cas12 collateral cleavage	Validated point-of-need diagnostics

7. Therapeutic translation and clinical evidence

7.1. Delivery: the rate-limiting step

The efficacy of any editing tool in vivo is gated by delivery. Three broad strategies dominate: viral vectors (principally adeno-associated virus, AAV), non-viral Lipid Nanoparticles (LNPs), and direct delivery of preassembled ribonucleoproteins (Wang *et al.*, 2020). Each embodies trade-offs. AAV offers efficient transduction of certain tissues but has limited cargo capacity and drives prolonged nuclease expression that may increase off-target risk and immunogenicity. LNPs enable transient, redosable, and manufacturable delivery, with a strong natural tropism for the liver that has made hepatic targets the vanguard of in vivo editing (Yin *et al.*, 2016; Finn *et al.*, 2018). Ribonucleoprotein delivery limits the duration of editor activity, improving specificity, and is well suited to ex vivo manufacturing (Zuris *et al.*, 2015). The dominant unmet need is efficient, selective delivery to extrahepatic tissues, which currently constrains the range of addressable diseases more than any limitation of the editors themselves.

7.2. Ex vivo cell therapies

Ex vivo editing sidesteps many delivery problems by manipulating cells outside the body under controlled conditions. Efficient editing of the β-globin locus and of the *BCL11A* erythroid enhancer in haematopoietic stem cells provided the scientific foundation for haemoglobinopathy therapies (Dever *et al.*, 2016; Wu *et al.*, 2019). In oncology, CRISPR has been used to disrupt endogenous checkpoints and to site-specifically integrate

chimeric antigen receptors, improving CAR T-cell function, while a first-in-human study established the feasibility and preliminary safety of multiplex-edited autologous T-cells in patients with refractory cancer (Eyquem *et al.*, 2017; Ren *et al.*, 2017; Stadtmauer *et al.*, 2020). An early report of *CCR5*-edited stem cells in a patient with HIV and leukaemia demonstrated durable engraftment of edited cells, albeit at a modest editing fraction, illustrating both promise and the challenge of achieving therapeutically sufficient editing (Xu *et al.*, 2019).

7.3. In vivo editing and regulatory approval

The translation of CRISPR into approved medicine has been swift by historical standards. The landmark demonstration that ex vivo editing to reactivate fetal haemoglobin could functionally cure transfusion-dependent β-thalassaemia and severe sickle cell disease led, after confirmatory trials, to the approval of exagamglogene autotemcel (exa-cel; Casgevy), the first CRISPR-based therapy authorised by a major regulator (Frangoul *et al.*, 2024). *In vivo* editing advanced in parallel: systemic LNP delivery of a Cas9–sgRNA system targeting *TTR* (NTLA-2001/nexiguran ziclumeran) produced deep, durable reductions in circulating transthyretin in patients with hereditary amyloidosis and, subsequently, in those with ATTR cardiomyopathy—the first convincing evidence that CRISPR can edit genes directly inside the human body (Gillmore *et al.*, 2021; Fontana *et al.*, 2024). Editing has also been delivered directly to the eye to address an inherited retinal degeneration (Maeder *et al.*, 2019). Perhaps the most striking recent milestone is the design, manufacture, and administration of a bespoke in vivo base-editing therapy for a single infant with a life-threatening urea-cycle disorder within months of diagnosis, signalling a plausible future of individualised "n-of-1" genetic medicines (Musunuru *et al.*, 2025). These successes are real, but they share a common feature—accessible target tissues (blood, liver, eye)—that reinforces how much remains contingent on solving delivery.

8. Recent innovations

Several converging innovations define the current frontier. First, the maturation of prime editing into a system capable of large, programmable structural changes (twin prime editing and related integrase-coupled approaches) is closing the gap between precise point correction and the insertion of whole therapeutic cassettes (Anzalone *et al.*, 2022). Second, *in vivo* base editing has moved from primate proof of concept to human application, with LNP-delivered editors achieving durable target modulation (Musunuru *et al.*, 2021; Frangoul *et al.*, 2024). Third, the enzyme discovery pipeline continues to yield compact and PAM-flexible effectors that ease delivery and broaden targeting, exemplified by near-PAMless Cas9 variants (Walton *et al.*, 2020). Fourth, CRISPR diagnostics matured into deployable platforms during a global health emergency (Broughton *et al.*, 2020). Finally, the demonstration of a rapidly manufactured, patient-specific therapy hints at a manufacturing and regulatory paradigm shift toward individualised medicines (Frangoul *et al.*, 2024). The unifying trend is a movement from generic tools toward disease- and even patient-tailored solutions.

9. Current challenges

The obstacles now facing the field are increasingly translational rather than purely molecular. *Specificity and genotoxicity* assessment must contend not only with small off-target indels but with large rearrangements, editor-specific nucleotide and RNA off-targets, and the confounding effects of the DNA damage response, all of which demand comprehensive, orthogonal assays rather than single-method reassurance. *Delivery* beyond the liver, blood, and eye remains the defining limitation for most indications (Doudna, 2020). *Immunogenicity*—both pre-existing adaptive immunity to bacterial Cas proteins and innate responses to delivery vehicles—may reduce efficacy and raise safety concerns, particularly for redosing (Charlesworth *et al.*, 2019). *Efficiency thresholds* differ by disease: some conditions require correction of only a small fraction of cells, whereas others demand near-complete editing, and current tools do not always reach the latter (Xu *et al.*, 2019). Finally, *equity and cost* loom large: therapies priced in the millions risk widening rather than narrowing health disparities for diseases such as sickle cell that are concentrated in under-resourced populations (Frangoul *et al.*, 2024).

10. Future perspectives

Near-term progress is likely to come from several directions. Engineered and newly discovered delivery vehicles—including tissue-tropic LNPs, redirected AAV capsids, and virus-like particles carrying preformed

editors—may extend *in vivo* editing to muscle, lung, and the central nervous system. Continued refinement of prime editors and their delivery could make versatile, break-free correction of the majority of pathogenic variants routine. Multiplexed and combinatorial editing will support polygenic and cell-engineering applications, while epigenome editing may offer durable yet reversible therapeutic modulation without permanent sequence change (Hilton *et al.*, 2015; Anzalone *et al.*, 2022). The individualised therapy paradigm, if it can be standardised and made affordable, could transform care for the long tail of ultra-rare diseases (Musunuru *et al.*, 2025). Realising these possibilities will require advances in manufacturing, regulatory science, and long-term safety surveillance to keep pace with molecular ingenuity.

11. Clinical implications

For clinicians, CRISPR is transitioning from a research curiosity to a therapeutic reality that will increasingly feature in practice. The approval of exa-cel establishes a template for one-time, potentially curative interventions in monogenic disease, with attendant considerations of conditioning toxicity, fertility, engraftment, and long-term monitoring that resemble those of stem-cell transplantation (Frangoul *et al.*, 2024). *In vivo* therapies such as *TTR* editing introduce a fundamentally new pharmacology—a single administration producing durable, potentially lifelong effects—that will demand new frameworks for consent, pharmacovigilance, and the management of irreversibility (Fontana *et al.*, 2024). The prospect of bespoke therapies for individual patients raises practical questions about equitable access, institutional readiness, and the evidentiary standards appropriate to n-of-1 interventions (Musunuru *et al.*, 2025). Clinicians will play a central role in patient selection, in communicating uncertainty, and in post-marketing surveillance for delayed adverse effects.

12. Research gaps

Several gaps warrant focused investigation. First, the true incidence and clinical consequence of large structural rearrangements and of p53-related selection during therapeutic editing remain inadequately quantified in human cells destined for the clinic (Kosicki *et al.*, 2018; Ihry *et al.*, 2018). Second, standardised, sensitive, and comparable off-target assays are lacking, hampering cross-study interpretation (Tsai *et al.*, 2017). Third, extrahepatic delivery is under-served by current technologies, and head-to-head comparisons of delivery modalities in relevant models are scarce (Wang *et al.*, 2020). Fourth, long-term durability, immunogenicity upon redosing, and the reversibility of epigenetic edits are insufficiently characterised. Fifth, the ethical, regulatory, and economic frameworks for germline governance and for equitable global access lag behind technical capability (Lander *et al.*, 2019). Addressing these gaps is a prerequisite for responsible scaling. A prioritised agenda is summarised in Table 5.

Table 5: Prioritised future research directions

Priority	Rationale	Illustrative goal
Extrahepatic delivery	Delivery gates most in vivo indications (Wang *et al.*, 2020)	Tissue-tropic vehicles for muscle, lung, CNS
Standardised genotoxicity assays	Cross-study comparability is lacking (Tsai *et al.*, 2017)	Consensus panel for on/off-target and structural variants
Efficient in vivo prime editing	Versatility limited by size/delivery (Anzalone *et al.*, 2019)	Compact editors; effective systemic delivery
Durable, reversible epigenetic therapy	Non-mutagenic modulation is attractive (Hilton *et al.*, 2015)	Stable yet reversible target silencing/activation
Equity, cost, and governance	Access risks widening disparities (Lander *et al.*, 2019)	Affordable manufacturing; global access frameworks

13. Conclusion

In little more than a decade, CRISPR-Cas systems have progressed from a mechanistic account of prokaryotic immunity to a diversified platform that reads, regulates, and rewrites nucleic acids, and that has now entered the clinic as approved and even individualised medicine. The trajectory has been driven by a virtuous cycle in which mechanistic understanding enabled engineering, and engineering exposed new mechanistic questions.

Base and prime editing addressed the liabilities of double-strand breaks; high-fidelity enzymes and unbiased off-target assays improved specificity; and delivery advances translated these tools into durable in vivo effects. Yet a sober assessment recognises that the field's remaining challenges—extrahepatic delivery, comprehensive genotoxicity assessment, immunogenicity, cost, and governance—are formidable and increasingly lie outside pure molecular biology. The decisive question for the coming decade is therefore not whether CRISPR can edit the genome precisely, which is largely settled, but whether the technology can be delivered, evaluated, and distributed safely and equitably at scale. Figure 4 situates these determinants within an integrative framework spanning discovery, tool development, and application.

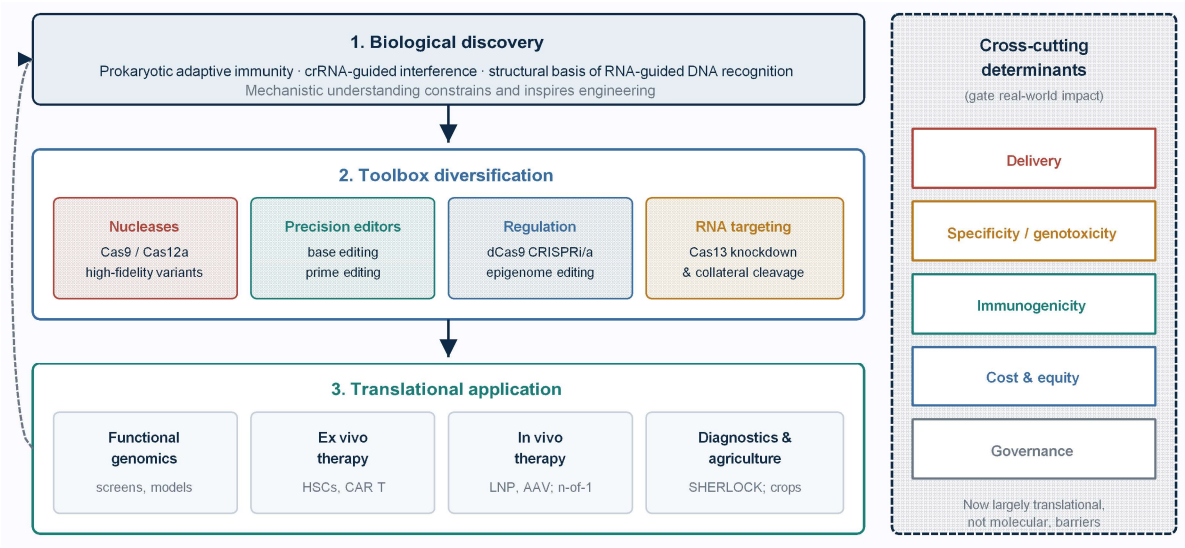

Figure 4: Conceptual framework linking discovery, tools, and impact. Mechanistic insight into prokaryotic adaptive immunity underpins a diversified toolbox—nucleases, precision editors, transcriptional and epigenetic regulators, and RNA-targeting effectors—which in turn enables application across functional genomics, ex vivo and in vivo therapeutics, diagnostics, and agriculture. Four cross-cutting determinants (delivery, specificity and genotoxicity, immunogenicity, cost and equity, and governance) gate the translation of technical capability into real-world benefit; these are now largely translational rather than molecular barriers. The dashed feedback arrow denotes the reciprocal loop in which engineering exposes new mechanistic questions

14. Key takeaways

- CRISPR-Cas originated as a prokaryotic adaptive immune system; its reprogrammability via RNA–DNA base pairing is the property that revolutionised genome engineering (Jinek *et al.*, 2012).
- Precision modalities—high-fidelity nucleases, base editors, and prime editors—progressively reduced reliance on double-strand breaks and homology-directed repair, each with distinct efficiency–versatility–specificity trade-offs (Gaudelli *et al.*, 2017; Anzalone *et al.*, 2019).
- Unintended effects, including large deletions, editor-specific off-targets, and DNA-damage-response selection, require orthogonal, comprehensive assessment rather than single-assay reassurance (Kosicki *et al.*, 2018; Zuo *et al.*, 2019).
- CRISPR extends beyond editing to transcriptional/epigenetic control, RNA targeting, and highly sensitive diagnostics (Qi *et al.*, 2013; Gootenberg *et al.*, 2017).
- Clinical translation is real: exa-cel is approved for haemoglobinopathies, in vivo *TTR* editing is durable, and the first patient-specific in vivo therapy has been administered (Frangoul *et al.*, 2024; Fontana *et al.*, 2024; Musunuru *et al.*, 2025).
- Delivery beyond accessible tissues, cost, equity, and governance are now the principal barriers to broader impact (Doudna, 2020; Lander *et al.*, 2019).

Conflict of interest statement

The author declares no competing financial or non-financial interests relevant to the content of this review.

Funding statement

This review received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgment

The author thanks colleagues whose discussions helped shape the scope and emphasis of this review. No third-party writing assistance was used in the preparation of the intellectual content.

References

Abudayyeh, O.O., Gootenberg, J.S., Essletzbichler, P., Han, S., Joung, J., Belanto, J.J. *et al.* (2017). RNA targeting with CRISPR-Cas13. *Nature*, 550(7675): 280-4.

Abudayyeh, O.O., Gootenberg, J.S., Konermann, S., Joung, J., Slaymaker, I.M., Cox, D.B.T. *et al.* (2016). C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, 353(6299): aaf5573.

Anzalone, A.V., Gao, X.D., Podracky, C.J., Nelson, A.T., Koblan, L.W., Raguram, A. *et al.* (2022). Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. *Nat Biotechnol*, 40(5): 731-40.

Anzalone, A.V., Koblan, L.W. and Liu, D.R. (2020). Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat Biotechnol*, 38(7): 824-44.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M. *et al.* (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576(7785): 149-57.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S. *et al.* (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, 315(5819): 1709-12.

Bolotin, A., Quinquis, B., Sorokin, A. and Ehrlich, S.D. (2005). Clustered Regularly Interspaced Short Palindrome Repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology*, 151(Pt 8): 2551-61.

Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J. *et al.* (2020). CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol*, 38(7): 870-4.

Brouns, S.J.J., Jore, M.M., Lundgren, M., Westra, E.R., Slijkhuis, R.J.H., Snijders, A.P.L. *et al.* (2008). Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science*, 321(5891): 960-4.

Casini, A., Olivieri, M., Petris, G., Montagna, C., Reginato, G., Maule, G. *et al.* (2018). A highly specific SpCas9 variant is identified by *in vivo* screening in yeast. *Nat Biotechnol*, 36(3): 265-71.

Charlesworth, C.T., Deshpande, P.S., Dever, D.P., Camarena, J., Lemgart, V.T., Cromer, M.K. *et al.* (2019). Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat Med*, 25(2): 249-54.

Chavez, A., Scheiman, J., Vora, S., Pruitt, B.W., Tuttle, M., Iyer, E.P.R. *et al.* (2015). Highly efficient Cas9-mediated transcriptional programming. *Nat Methods*, 12(4): 326-8.

Chen, J.S., Dagdas, Y.S., Kleinstiver, B.P., Welch, M.M., Sousa, A.A., Harrington, L.B. *et al.* (2017). Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. *Nature*, 550(7676): 407-10.

Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M. *et al.* (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, 360(6387): 436-9.

Chen, K., Wang, Y., Zhang, R., Zhang, H. and Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annu Rev Plant Biol*, 70: 667-97.

Chen, P.J., Hussmann, J.A., Yan, J., Knipping, F., Ravisankar, P., Chen, P.F. *et al.* (2021). Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. *Cell*, 184(22): 5635-52.

Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N. *et al.* (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, 339(6121): 819-23.

Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Joung, J. *et al.* (2017). RNA editing with CRISPR-Cas13. *Science*, 358(6366): 1019-27.

Deltcheva, E., Chylinski, K., Sharma, C.M., Gonzales, K., Chao, Y., Pirzada, Z.A. *et al.* (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*, 471(7340): 602-7.

Dever, D.P., Bak, R.O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C.E. *et al.* (2016). CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature*, 539(7629): 384-9.

Doench, J.G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E.W., Donovan, K.F. *et al.* (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol*, 34(2): 184-91.

Doudna, J.A. and Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213): 1258096.

Doudna, J.A. (2020). The promise and challenge of therapeutic genome editing. *Nature*, 578(7794): 229-36.

Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S.J.C., Hamieh, M., Cunanan, K.M. *et al.* (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature*, 543(7643): 113-7.

Finn, J.D., Smith, A.R., Patel, M.C., Shaw, L., Youniss, M.R., van Heteren, J. *et al.* (2018). A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Rep*, 22(9): 2227-35.

Fontana, M., Solomon, S.D., Kachadourian, J., Walsh, L., Rocha, R., Lebwohl, D. *et al.* (2024). CRISPR-Cas9 gene editing with nexiguran ziclumeran for ATTR cardiomyopathy. *N Engl J Med*, 391(23): 2231-41. doi:10.1056/NEJMoa2412309.

Frangoul, H., Altshuler, D., Cappellini, M.D., Chen, Y.S., Domm, J., Eustace, B.K. *et al.* (2021). CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *N Engl J Med*, 384(3): 252-60.

Frangoul, H., Locatelli, F., Sharma, A., Bhatia, M., Mapara, M., Molinari, L. *et al.* (2024). Exagamglogene autotemcel for severe sickle cell disease. *N Engl J Med*, 390(18): 1649-62.

Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K. *et al.* (2013). High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*, 31(9): 822-6.

Fu, Y., Sander, J.D., Reyon, D., Cascio, V.M. and Joung, J.K. (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nat Biotechnol*, 32(3): 279-84.

Gao, C. (2021). Genome engineering for crop improvement and future agriculture. *Cell*, 184(6): 1621-35.

Garneau, J.E., Dupuis, M.È., Villion, M., Romero, D.A., Barrangou, R., Boyaval, P. *et al.* (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature*, 468(7320): 67-71.

Gasiunas, G., Barrangou, R., Horvath, P. and Siksnys, V. (2012). Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc Natl Acad Sci USA*, 109(39): E2579-86.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I. *et al.* (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, 551(7681): 464-71.

Gilbert, L.A., Larson, M.H., Morsut, L., Liu, Z., Brar, G.A., Torres, S.E. *et al.* (2013). CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*, 154(2): 442-51.

Gillmore. J.D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M.L. *et al.* (2021). CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med*, 385(6): 493-502. doi:10.1056/NEJMoa2107454.

Gootenberg, J.S., Abudayyeh, O.O., Kellner, M.J., Joung, J., Collins, J.J. and Zhang, F. (2018). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*, 360(6387): 439-44.

Gootenberg, J.S., Abudayyeh, O.O., Lee, J.W., Essletzbichler, P., Dy, A.J., Joung, J. *et al.* (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, 356(6336): 438-42.

Grünewald, J., Zhou, R., Garcia, S.P., Iyer, S., Lareau, C.A., Aryee, M.J. *et al.* (2019). Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. *Nature*, 569(7756): 433-7.

Haapaniemi, E., Botla, S., Persson, J., Schmierer, B. and Taipale, J. (2018). CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nat Med*, 24(7): 927-30.

Hilton, I.B., D'Ippolito, A.M., Vockley, C.M., Thakore, P.I., Crawford, G.E., Reddy, T.E. *et al.* (2015). Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat Biotechnol*, 33(5): 510-7.

Hsu, P.D., Scott, D.A., Weinstein, J.A., Ran, F.A., Konermann, S., Agarwala, V. *et al.* (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol.*, 31(9): 827-32.

Hu, J.H., Miller, S.M., Geurts, M.H., Tang, W., Chen, L., Sun, N. *et al.* (2018). Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature*, 556(7699): 57-63.

Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K. *et al.* (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. *Nat Med*, 24(7): 939-46.

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M. and Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J Bacteriol*, 169(12): 5429-33.

Jiang, F. and Doudna, J.A. (2017). CRISPR-Cas9 structures and mechanisms. *Annu Rev Biophys*, 46: 505-29.

Jin, S., Zong, Y., Gao, Q., Zhu, Z., Wang, Y., Qin, P. *et al.* (2019). Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science*, 364(6437): 292-5.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096): 816-21.

Kantor, A., McClements, M.E. and MacLaren, R.E. (2020). CRISPR-Cas9 DNA base-editing and prime-editing. *Int J Mol Sci*, 21(17): 6240.

Kellner, M.J., Koob, J.G., Gootenberg, J.S., Abudayyeh, O.O. and Zhang, F. (2019). SHERLOCK: Nucleic acid detection with CRISPR nucleases. *Nat Protoc*, 14(10): 2986-3012.

Kleinstiver, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z. *et al.* (2016). High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, 529(7587): 490-5.

Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Topkar, V.V., Nguyen, N.T., Zheng, Z. *et al.* (2015). Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature*, 523(7561): 481-5.

Knott, G.J. and Doudna, J.A. (2018). CRISPR-Cas guides the future of genetic engineering. *Science*, 361(6405): 866-9.

Koblan, L.W., Doman, J.L., Wilson, C., Levy, J.M., Tay, T., Newby, G.A. *et al.* (2018). Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. *Nat Biotechnol*, 36(9): 843-6.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A. and Liu, D.R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 533(7603): 420-4.

Konermann, S., Brigham, M.D., Trevino, A.E., Joung, J., Abudayyeh, O.O., Barcena, C. *et al.* (2015). Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*, 517(7536): 583-8.

Konermann, S., Lotfy, P., Brideau, N.J., Oki, J., Shokhirev, M.N. and Hsu, P.D. (2018). Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell*, 173(3): 665-76.

Koonin, E.V. and Makarova, K.S. (2019). Origins and evolution of CRISPR-Cas systems. *Philos Trans R Soc Lond B Biol Sci*, 374(1772): 20180087.

Kosicki, M., Tomberg, K. and Bradley, A. (2018). Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol*, 36(8): 765-71.

Lander, E.S., Baylis, F., Zhang, F., Charpentier, E., Berg, P., Bourgain, C. *et al.* (2019). Adopt a moratorium on heritable genome editing. *Nature*, 567(7747): 165-8.

Maeder, M.L., Stefanidakis, M., Wilson, C.J., Baral, R., Barrera, L.A., Bounoutas, G.S. *et al.* (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nat Med*, 25(2): 229-33.

Makarova, K.S., Wolf, Y.I., Iranzo, J., Shmakov, S.A., Alkhnbashi, O.S., Brouns, S.J.J. *et al.* (2020). Evolutionary classification of CRISPR-Cas systems: A burst of class 2 and derived variants. *Nat Rev Microbiol*, 18(2): 67-83.

Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E. *et al.* (2013). RNA-guided human genome engineering via Cas9. *Science*, 339(6121): 823-6.

Marraffini, L.A. and Sontheimer, E.J. (2008). CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Science*, 322(5909): 1843-5.

Mojica, F.J.M., Díez-Villaseñor, C., García-Martínez, J. and Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol.*, 60(2): 174-82.

Musunuru, K., Chadwick, A.C., Mizoguchi, T., Garcia, S.P., DeNizio, J.E., Reiss, C.W. *et al.* (2021). In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. *Nature*, 593(7859): 429-34.

Musunuru, K., Grandinette, S.A., Wang, X., Hudson, T.R., Briseno, K., Berry, A.M. *et al.* (2025). Patient-specific in vivo gene editing to treat a rare genetic disease. *N Engl J Med*, 392(22): 2235-43. doi:10.1056/NEJMoa2504747.

Nelson, J.W., Randolph, P.B., Shen, S.P., Everette, K.A., Chen, P.J., Anzalone, A.V. *et al.* (2022). Engineered pegRNAs improve prime editing efficiency. *Nat Biotechnol*, 40(3): 402-10.

Newby, G.A., Yen, J.S., Woodard, K.J., Mayuranathan, T., Lazzarotto, C.R., Li, Y. *et al.* (2021). Base editing of haematopoietic stem cells rescues sickle cell disease in mice. *Nature*, 595(7866): 295-302.

Nishimasu, H., Ran, F.A., Hsu, P.D., Konermann, S., Shehata, S.I., Dohmae, N. *et al.* (2014). Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell*, 156(5): 935-49.

Pickar-Oliver, A. and Gersbach, C.A. (2019). The next generation of CRISPR-Cas technologies and applications. *Nat Rev Mol Cell Biol*, 20(8): 490-507.

Platt, R.J., Chen, S., Zhou, Y., Yim, M.J., Swiech, L., Kempton, H.R. *et al.* (2014). CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell*, 159(2): 440-55.

Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P. *et al.* (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, 152(5): 1173-83.

Ran, F.A., Hsu, P.D., Lin, C.Y., Gootenberg, J.S., Konermann, S., Trevino, A.E. *et al.* (2013). Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell*, 154(6): 1380-9.

Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A. and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*, 8(11): 2281-308.

Rees, H.A. and Liu, D.R. (2018). Base editing: Precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet*, 19(12): 770-88.

Ren, J., Liu, X., Fang, C., Jiang, S., June, C.H. and Zhao, Y. (2017). Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. *Clin Cancer Res*, 23(9): 2255-66.

Rothgangl, T., Dennis, M.K., Lin, P.J.C., Oka, R., Witzigmann, D., Villiger, L. *et al.* (2021). In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol. *Nat Biotechnol*, 39(8): 949-57.

Schene, I.F., Joore, I.P., Oka, R., Mokry, M., van Vugt, A.H.M., van Boxtel, R. *et al.* (2020). Prime editing for functional repair in patient-derived disease models. *Nat Commun*, 11(1): 5352.

Shalem, O., Sanjana, N.E., Hartenian, E., Shi, X., Scott, D.A., Mikkelson, T. *et al.* (2014). Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*, 343(6166): 84-7.

Slaymaker, I.M., Gao, L., Zetsche, B., Scott, D.A., Yan, W.X. and Zhang, F. (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science*, 351(6268): 84-8.

Stadtmauer, E.A., Fraietta, J.A., Davis, M.M., Cohen, A.D., Weber, K.L., Lancaster, E. *et al.* (2020). CRISPR-engineered T cells in patients with refractory cancer. *Science*, 367(6481): eaba7365.

Sternberg, S.H., Redding, S., Jinek, M., Greene, E.C. and Doudna, J.A. (2014). DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature*, 507(7490): 62-7.

Tsai, S.Q., Nguyen, N.T., Malagon-Lopez, J., Topkar, V.V., Aryee, M.J. and Joung, J.K. (2017). CIRCLE-seq: A highly sensitive in vitro screen for genome-wide CRISPR-Cas9 nuclease off-targets. *Nat Methods*, 14(6): 607-14.

Tsai, S.Q., Zheng, Z., Nguyen, N.T., Liebers, M., Topkar, V.V., Thapar, V. *et al.* (2015). GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol*, 33(2): 187-97.

Walton, R.T., Christie, K.A., Whittaker, M.N. and Kleinstiver, B.P. (2020). Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. *Science*, 368(6488): 290-6.

Wang, D., Zhang, F. and Gao, G. (2020). CRISPR-based therapeutic genome editing: Strategies and in vivo delivery by AAV vectors. *Cell*, 181(1): 136-50.

Wang, T., Wei, J.J., Sabatini, D.M. and Lander, E.S. (2014). Genetic screens in human cells using the CRISPR-Cas9 system. *Science*, 343(6166): 80-4.

Wu, Y., Zeng, J., Roscoe, B.P., Liu, P., Yao, Q., Lazzarotto, C.R. *et al.* (2019). Highly efficient therapeutic gene editing of human hematopoietic stem cells. *Nat Med*, 25(5): 776-83.

Xu, L., Wang, J., Liu, Y., Xie, L., Su, B., Mou, D. *et al.* (2019). CRISPR-edited stem cells in a patient with HIV and acute lymphocytic leukemia. *N Engl J Med*, 381(13): 1240-7.

Yin, H., Song, C.Q., Dorkin, J.R., Zhu, L.J., Li, Y., Wu, Q. *et al.* (2016). Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. *Nat Biotechnol*, 34(3): 328-33.

Zhang, Y., Massel, K., Godwin, I.D. and Gao, C. (2018). Applications and potential of genome editing in crop improvement. *Genome Biol*, 19(1): 210.

Zuo, E., Sun, Y., Wei, W., Yuan, T., Ying, W., Sun, H. *et al.* (2019). Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. *Science*, 364(6437): 289-92.

Zuris, J.A., Thompson, D.B., Shu, Y., Guilinger, J.P., Bessen, J.L., Hu, J.H. *et al.* (2015). Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. *Nat Biotechnol*, 33(1): 73-80.

Cite this article as: Florento Eric Zkayi (2026). Recent advances in CRISPR-Cas systems and their applications in biological research. *Biomed Pharma Reviews. 2(2)*, 50-65. doi: 10.62587/BMPR.2.2.2026.50-65.