CRISPR-Cas in translational research: From molecular mechanisms to therapeutic applications in human disease (Review)

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Abstract. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) systems are transformative tools in biomedical research, enabling precise and programmable manipulation of genetic material. Originally identified as adaptive immune mechanisms in bacteria and archaea, CRISPR-Cas technologies have evolved into versatile platforms for genome editing, disease modeling, molecular diagnostics and therapeutic development. Their simplicity, efficiency and adaptability have accelerated applications across a wide range of human diseases, including inherited genetic disorders, infectious disease, cancer and immune-mediated conditions. The present review provides a comprehensive overview of the molecular mechanisms, classification and evolution of CRISPR-Cas systems, with particular emphasis on their translational applications in modern medicine. The present review aimed to summarize advances in disease modeling, functional genomic screening, diagnostic platforms and therapeutic genome editing, as well as the clinical progress of CRISPR-based interventions, including *ex vivo* cell therapies and emerging *in vivo* gene editing approaches, while distinguishing their relative stages of clinical maturity. Beyond therapeutic opportunities, challenges continue to limit the widespread clinical implementation of CRISPR technologies. Key barriers include off-target genome modification, inefficient delivery to specific tissue, limitations in large-fragment genome editing, immunogenicity of bacterial-derived Cas proteins, manufacturing and scalability constraints and uncertainties regarding long-term safety. The present review aimed to summarize ethical and regulatory considerations associated with human genome editing. The present review highlighted both the promise and the remaining challenges of CRISPR-Cas systems. Continued progress in editing precision, delivery technology, safety assessment and regulatory governance will be essential for realizing the full clinical potential of genome editing therapies.

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1. Introduction

Advances in genome editing technologies have expanded the ability to investigate gene function and develop novel therapeutic strategies for human diseases; among these technologies, the Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) system has emerged as one of the most efficient and versatile tools for targeted genome modification (1). The system was originally identified as an adaptive immune mechanism in bacteria and archaea, where it protects host cells against invading bacteriophages and foreign plasmid DNA through sequence-specific recognition and cleavage of nucleic acids (1). The adaptation of CRISPR-Cas systems for genome engineering has enabled precise and programmable manipulation of genetic sequences across diverse organisms and cell types (2).

The most commonly used CRISPR platform is the CRISPR-Cas9 system derived from *Streptococcus pyogenes*. This system uses a single guide RNA (sgRNA), formed by the fusion of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA), to direct the Cas9 endonuclease to a complementary DNA sequence in the genome (2). Upon recognition of a protospacer adjacent motif (PAM), Cas9 introduces a double-strand break (DSB) at the target site. The break is repaired by endogenous cellular mechanisms, primarily non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling targeted gene disruption, insertion or correction. Because of its simplicity, high efficiency and adaptability, CRISPR-Cas9 has rapidly become a central tool in molecular biology and biomedical research (3). The application of CRISPR-based genome editing has contributed to advances across genetic diseases, cancer biology and infectious diseases. The system has enabled gene correction for monogenic disorders including hemoglobinopathy, cystic fibrosis and hereditary tyrosinemia, while also facilitating the generation of disease models in cultured and induced pluripotent stem cells and animal systems (4). In oncology, CRISPR screening approaches have identified genes involved in tumor development, drug resistance and immune evasion. Beyond therapeutic applications, CRISPR systems have been adapted for molecular diagnostics; platforms such as Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) and DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) use CRISPR enzymes to detect pathogen-derived nucleic acids with high sensitivity and specificity, with clinical validation for SARS-CoV-2, Zika and Dengue virus detection. Despite these advances, challenges remain for the clinical use of CRISPR-based technology. Off-target genome editing, efficient delivery of CRISPR components into specific tissue, pre-existing and adaptive immune responses to Cas proteins and ethical considerations associated with germline modification require careful evaluation (5). The present review aimed to summarize CRISPR-Cas systems, including their discovery, molecular mechanisms and classification, with emphasis on translational research and potential clinical applications in human disease.

2. Molecular architecture of CRISPR-Cas systems

Discovery and evolutionary origins. The transition of prokaryotic CRISPR-Cas adaptive immune networks into programmable molecular technology represents a foundational milestone in genetic medicine (1). Historically, while hyper-variable, regularly repeated DNA sequences interspaced by unique spacer hallmarks were first documented in the *Escherichia coli* genome during the late 1980s, their physiological utility remained an enigma (6). Systematic *in silico* evaluations by (7) confirmed the ubiquity of these palindromic repeats across diverse prokaryotic genomes, officially introducing the CRISPR nomenclature and identifying an adjacent, highly conserved cluster of helicase and nuclease genes designated as *Cas* loci. (8) demonstrated that these hyper-variable spacer sequences share near-absolute homology with bacteriophage genomes and exogenous plasmid DNA vectors. This sequence-specific convergence provided definitive evidence that the CRISPR platform serves as a highly dynamic, heritable adaptive immune system, facilitating memory-guided nucleic acid restriction to preserve prokaryotic genomic integrity against viral selective pressure (9). Genomic and metagenomic profiling has since validated that functional CRISPR-Cas loci are encoded within ~45% of bacterial genomes and 90% of archaeal lineages (10). This widespread ecological distribution highlights the evolutionary success of RNA-guided defense mechanics and simultaneously presents a major clinical hurdle: The extreme evolutionary conservation of these microbial effectors means modern human populations exhibit high rates of pre-existing adaptive immunity against the nucleases adapted for therapeutic translation.

Structural classification and effector topology: Translational implications. The phylogenetic taxonomy of CRISPR-Cas systems divides them into structurally distinct classes with fundamentally different translational trajectories (11). This dichotomy, rather than any inherent functional superiority, explains why Class 2 systems dominate clinical development due to simpler single-effector architecture and reduced delivery requirements, whereas Class 1 systems require multi-subunit complexes that complicate therapeutic translation, while Class 1 systems remain confined to basic research and microbial engineering (Fig. 1; Table I).

Class 1 systems (types I, III and IV). Class 1 systems employ multi-subunit effector complexes for surveillance and interference. The universally conserved Cas1-Cas2 complex mediates spacer acquisition, while specialized multi-protein assemblies such as the type I cascade complex execute crRNA-guided target recognition following maturation by endoribonucleases including Cas5 and Cas6 (12). Type I systems recruit the trans-acting helicase-nuclease Cas3 to propagate processive, multi-kilobase deletions. While this long-range erasure capability is key for functional genomics and microbial engineering, the requirement for stoichiometrically balanced expression of 5-8 distinct protein subunits imposes a substantial delivery burden for *in vivo* somatic gene therapy (13). The physical cargo capacity of adeno-associated virus (AAV) vectors, the clinical gold standard for *in vivo* delivery, is limited to ~4.7 kilobases (kb). Even the smallest known Class 1 system requires >6 kb coding sequence, precluding single-vector packaging. Dual-AAV strategies demonstrate <5% co-transduction efficiency in

Table I. Comparative platform analysis.

Feature	Type II (Cas9)	Type V (Cas12)	Type VI (Cas13)
Target	DNA (ds)	DNA (ds)	RNA (ss)
DSB type	Blunt-ended	Staggered (5-10 nt overhangs)	No DSB (RNA cleavage)
Collateral activity	No	Yes (ssDNA)	Yes (ssRNA)
Size, kb	3.3 (SaCas9)-4.2 (SpCas9)	3.5-4.0	3.0-3.5
Multiplex capability	Requires multiple sgRNAs	Single crRNA array	Single crRNA array
Primary application	Therapeutic editing	Diagnostics (DETECTR)	Diagnostics (SHERLOCK)
Regulatory status	Approved	Research/EUA	Research/EUA

Cas, CRISPR-associated protein; ds, double-stranded; ss, single-stranded; DSB, double-strand break; nt, nucleotide; Sa, *Staphylococcus aureus*; Sp, Streptococcus pyogenes; sg, single-guide; cr, CRISPR; DETECTR, DNA Endonuclease-Targeted CRISPR Trans Reporter; SHERLOCK, Specific High-Sensitivity Enzymatic Reporter Unlocking; EUA, Emergency Use Authorization; SCD, Sickle Cell Disease; TDT, Transfusion-Dependent β-Thalassemia.

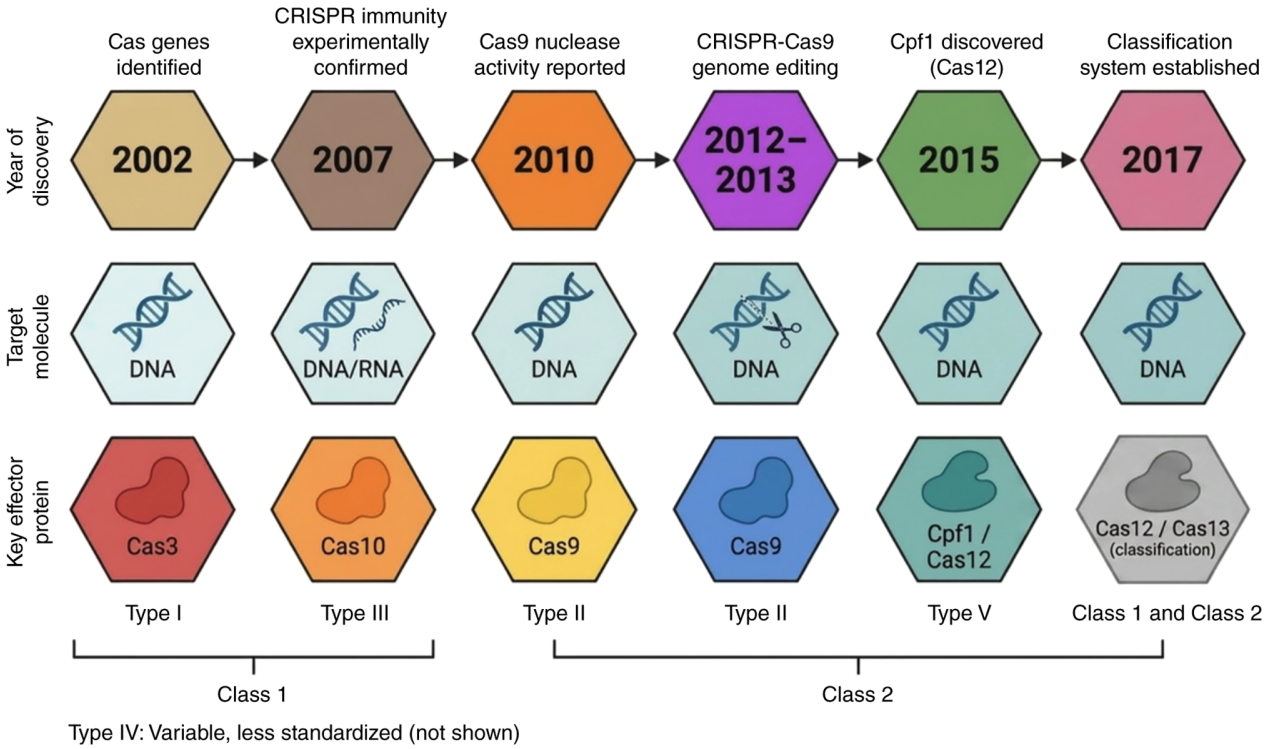


Figure 1. Historical development and classification of CRISPR-Cas systems. CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; Cpf, CRISPR from Prevotella and Francisella.

most tissue (14). To the best of our knowledge, no Class 1 system has entered clinical trials for direct *in vivo* genome editing (15).

Class 2 systems (types II, V and VI). In Class 2 CRISPR-Cas systems, all necessary functional domains responsible for genomic scanning, sequence-specific target identification, and phosphodiester bond hydrolysis are integrated into a single, multifunctional effector complex. This structural minimalism decreases the genetic cargo requirement to a single open reading frame (typically 3.0-4.5 kb), fitting within AAV packaging limits and enabling delivery as mRNA, plasmid DNA or pre-assembled RNP complexes (16,17).

Type II (Cas9 platforms). Cas9 nucleases introduce blunt-ended DSBs through coordinated HNH (target strand) and RuvC (non-target strand) domain activity. S. pyogenes Cas9 (SpCas9; 4.2 kb, 1,368 amino acids) recognizes the canonical NGG protospacer adjacent motif (PAM) and remains the most extensively characterized and widely used platform (18,19). Staphylococcus aureus Cas9 (SaCas9; 3.3 kb, 1,053 amino acids) recognizes NNGRRT PAM and is preferred for AAV delivery due to its smaller size (20). Exagamglogene autotemcel, sold under the brand name Casgevy, an *ex vivo* CRISPR-Cas9-edited hematopoietic stem cell therapy, received US Food and Drug Administration (FDA) approval for sickle cell disease in December 2023

and transfusion-dependent β-thalassemia in January 2024, becoming the first approved CRISPR-based therapy (21,22).

Type V (Cas12 platforms). Cas12 endonucleases (Cas12a, Cas12b, Cas12i) contain a single RuvC domain that processes crRNA arrays, enabling efficient multiplex gene editing without requiring tracrRNA. Upon target recognition, Cas12 generates staggered, cohesive-ended DSBs with 5-10 nucleotide 5' overhangs. These overhangs favor microhomology-mediated end joining (MMEJ) over error-prone blunt-end NHEJ, potentially improving precise repair outcomes (23). Upon target binding, Cas12 undergoes a conformational shift that triggers non-specific, collateral cleavage of surrounding single-stranded DNA. For *in vivo* therapeutics, this indiscriminate trans-cleavage activity causes cell toxicity and off-target genomic damage, rendering Cas12 unsuitable for therapeutic editing (24). However, this mechanism enables signal amplification for diagnostic applications, serving as the biochemical foundation for the DETECTR platform, which achieves attomolar sensitivity without pre-amplification (25).

Type VI (Cas13 platforms). Cas13 nucleases (Cas13a, Cas13b, Cas13d) operate as programmable RNA-guided RNA-targeting nucleases through dual higher eukaryotes and prokaryotes nucleotide-binding higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains. Unlike Cas9 or Cas12, Cas13 has no detectable effect on DNA, making it uniquely suitable for transcriptome engineering and transient gene silencing without permanent genomic modification (26). Upon target RNA recognition, Cas13 activates HEPN-mediated collateral cleavage of surrounding single-stranded RNA in a non-sequence-specific manner. For therapeutic applications, this trans-cleavage activity degrades non-target cell mRNAs, causing transcriptomic instability and potential cytotoxicity. Consequently, Cas13 has not advanced into therapeutic clinical trials for *in vivo* applications (27). The collateral RNA cleavage activity enables exponential signal amplification, forming the biochemical basis for SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing). SHERLOCK achieves attomolar (10^{-18} M) sensitivity for RNA virus detection, including SARS-CoV-2, Zika, and Dengue, with single-base specificity. The platform received FDA Emergency Use Authorization for coronavirus disease 2019 (COVID-19) diagnostics in 2020 (28,29).

Biochemical kinetics and core molecular components. From a mechanistic perspective, the operational fidelity of the prototype class 2 CRISPR platform utilizes a strictly coordinated bi-component architecture composed of a single effector endonuclease (Cas9) complexed with an engineered sgRNA architecture (30). In its native state, this guide system serves as a ribonucleoprotein (RNP) duplex formed by the hybridization of a target-specifying crRNA and a structurally stabilizing tracrRNA (31). The co-engineered fusion of these elements into a single-molecule sgRNA chimeric transcript has streamlined the packaging parameters required for somatic engineering (32).

The molecular cascade governing targeted DSB induction proceeds via a sequential molecular recognition and cleavage process involving PAM recognition, R-loop formation and coordinated nuclease cleavage. The RNP complex executes rapid, non-target-directed three-dimensional diffusion across the host genome, hunting for a short, locus-specific PAM. For *S. pyogenes* Cas9, this canonical sequence manifests as a 5'NGG3' motif, recognized via major-groove readout by the C-terminal PAM-interacting domain of the nuclease (33). Binding to the PAM triggers local duplex destabilization and drives processive directional unzipping of the genomic DNA. This allows the 20-nucleotide target-specifying seed region of the sgRNA to evaluate complementarity with the incoming target strand, culminating in the assembly of a stable R-loop conformation (34). Upon precise R-loop validation, the structurally decoupled HNH domain (target-strand cleaving) and RuvC domain (non-target-strand cleaving) catalytic domains execute coordinated, magnesium-dependent phosphodiester backbone hydrolysis three base pairs upstream of the PAM boundary. The HNH domain cuts the complementary target strand, while the RuvC domain cleaves the non-complementary strand, generating clean, blunt-ended DSBs (Table II) (16). Understanding these biochemical kinetics is key for translational research. Because Cas9 relies predominantly on the short seed region to drive R-loop stabilization, the nuclease is prone to tolerating single or multiple nucleotide mismatches within the distal non-seed region of the protospacer. This kinetic tolerance directly induces off-target effects and unexpected genotoxicity across partially homologous genomic loci, a technical barrier that requires active mitigation via the engineering of high-fidelity variants or transient delivery strategies to safeguard human genome integrity (35).

3. CRISPR-Cas systems in disease modeling

Applications of CRISPR-Cas systems in the development of human disease animal and cell models. CRISPR-Cas genome editing has been applied in biological systems, including microbes, plants and animals. Studies have reported successful genome editing in numerous species such as *Drosophila*, zebrafish, yeast, *Arabidopsis*, pigs, mice, sheep and non-human primates (19,36,37). The technology has also been used in human cell lines, including tumor and stem cells (38). These applications have expanded the ability to investigate disease mechanisms and evaluate therapeutic interventions (39).

CRISPR-Cas9 has enabled the development of experimental disease models in both cell systems and whole organisms. These models allow researchers to reproduce genetic mutations associated with human disease and to analyze their biological effects. Genome editing approaches have been used to study monogenic disorders such as sickle cell disease, as well as complex diseases, including Alzheimer's disease (40). Examples of CRISPR-based disease models are summarized in Table III.

Development of animal models of human disease. Animal models serve an essential role in biomedical research and are widely used to study the mechanisms of human disease. Among these organisms, mice are used because of their genetic similarity to humans and the availability of genetic tools (41). CRISPR-Cas9 technology has simplified the generation of genetically modified mouse models by enabling targeted mutations to be introduced directly into embryos (42). In addition to mice, CRISPR genome editing has been applied to generate disease models in larger animals, including rabbits, pigs and non-human primates, which provide additional information

Table II. Classification and characteristics of CRISPR-Cas systems.

Class	Type	Major subtypes	Representative/ signature effector	Effector architecture	Principal target	Key characteristic
Class 1	I	I-A-I-G, I-F variants*	Cas3 + Cascade complex	Multi-subunit	DNA	Cascade recognizes target DNA and recruits Cas3 for processive DNA degradation
Class 1	III	III-A-III-I*	Cas10-containing Csm/Cmr complex	Multi-subunit	RNA; transcription-associated DNA	RNA targeting combined with Cas10-mediated interference and signaling
Class 1	IV	IV-A-IV-C*	Csf-associated effector complex	Multi-subunit	DNA/mobile genetic elements	Typically associated with plasmids and other mobile genetic elements
Class 1	VII	VII-A	Cas14 with Cas5/Cas7 complex	Multi-subunit	RNA	Cas14 functions as the signature RNase effector for target RNA cleavage
Class 2	II	II-A-II-D	Cas9	Single multidomain effector	DNA	RNA-guided DNA cleavage through HNH and RuvC nuclease domains
Class 2	V	Multiple Cas12-associated subtypes	Cas12	Single multidomain effector	DNA	RuvC-mediated DNA cleavage; several Cas12 enzymes exhibit collateral ssDNA cleavage
Class 2	VI	Cas13-associated subtypes	Cas13	Single multidomain effector	RNA	HEPN-domain-mediated RNA cleavage; collateral RNA cleavage is exploited in diagnostics

CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; Csm, CRISPR-Cas subtype III-A effector complex; Cmr, CRISPR-Cas subtype III-B effector complex; Csf, CRISPR-Cas type IV-associated effector complex; ssDNA, single-stranded DNA; HNH, HNH nuclease domain; RuvC, RuvC nuclease domain; HEPN, higher eukaryotes and prokaryotes nucleotide-binding domain.

that may not be captured in rodent systems, particularly for diseases involving complex physiology (43). CRISPR-based knock-in is used in pigs to investigate neurodegenerative disorders such as Huntington's disease (38).

Advances in genome editing have enabled the development of primate models for neurological disease (44). CRISPR-edited marmosets have been used to examine the genetic basis of neurodegenerative disorder, including Parkinson's disease (45). The development of such models provides opportunities to study disease progression and evaluate therapeutic strategies (46).

Development of next-generation human disease cell and scaffold-based models. The evolution of disease modeling has transitioned from traditional 2D monochromatic cell cultures to structurally layered, multi-cellular biomimetic platforms (47). This structural scaling is facilitated by the convergence of CRISPR-Cas functional genome editing and state-of-the-art tissue engineering. Pooled genetic screens using patient-derived induced pluripotent stem cells are now routinely deployed on three-dimensional cancer spheroids and patient-matched organoids (including skin, heart, cartilage, liver, lung, pancreas and the central nervous system), providing high-fidelitymodels (48), that accurately recapitulate donor-specific drug responses and resistance networks (49).

CRISPR-edited cells are encapsulated in hydrogels and decellularized extracellular matrix composites via bioprinting, while 4D bioprinting enables temporal control over multi-cellular structures, facilitating target validation before clinical trials (50). This kinetic modeling facilitates time-sensitive analyses of cell biophysical dynamics and cell-to-matrix interactions under precise mechanical control, serving as a platform for target validation and screening algorithms before moving into human-phase clinical trials (51,52).

Advantages of CRISPR/Cas9 for cell and animal model development. CRISPR-Cas9 technology provides advantages compared with earlier genome editing methods such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). One advantage is that target specificity is determined by the gRNA sequence rather than by protein engineering (53). CRISPR systems allow multiplex genome editing, meaning that several genes can be targeted simultaneously using multiple gRNAs. This makes it possible to generate complex genetic models in a shorter time frame (24). Traditional embryonic stem cell-based approaches may require >1 year to produce modified animals. By contrast,

Table III. Applications of clustered regularly interspaced short palindromic repeats-associated protein 9 genome editing in disease models.

Disease model	Animal/cells	Gene target	Outcome
Sickle cell disease	Mouse, human stem cells	HBB	Correction of mutation and restoration of normal hemoglobin
Cystic fibrosis	Human airway epithelial cells	CFTR	Restoration of chloride channel activity
Alzheimer's disease	Mouse, human neurons	APP, PSEN1	Decreased amyloid-related pathology
Cancer (T cell therapy)	Human T cells	TCR, PD-1	Enhanced immune response against tumor cells

HBB, hemoglobin subunit beta; CFTR, cystic fibrosis transmembrane conductance regulator; APP, amyloid precursor protein; PSEN1, presenilin-1; TCR, T-cell receptor.

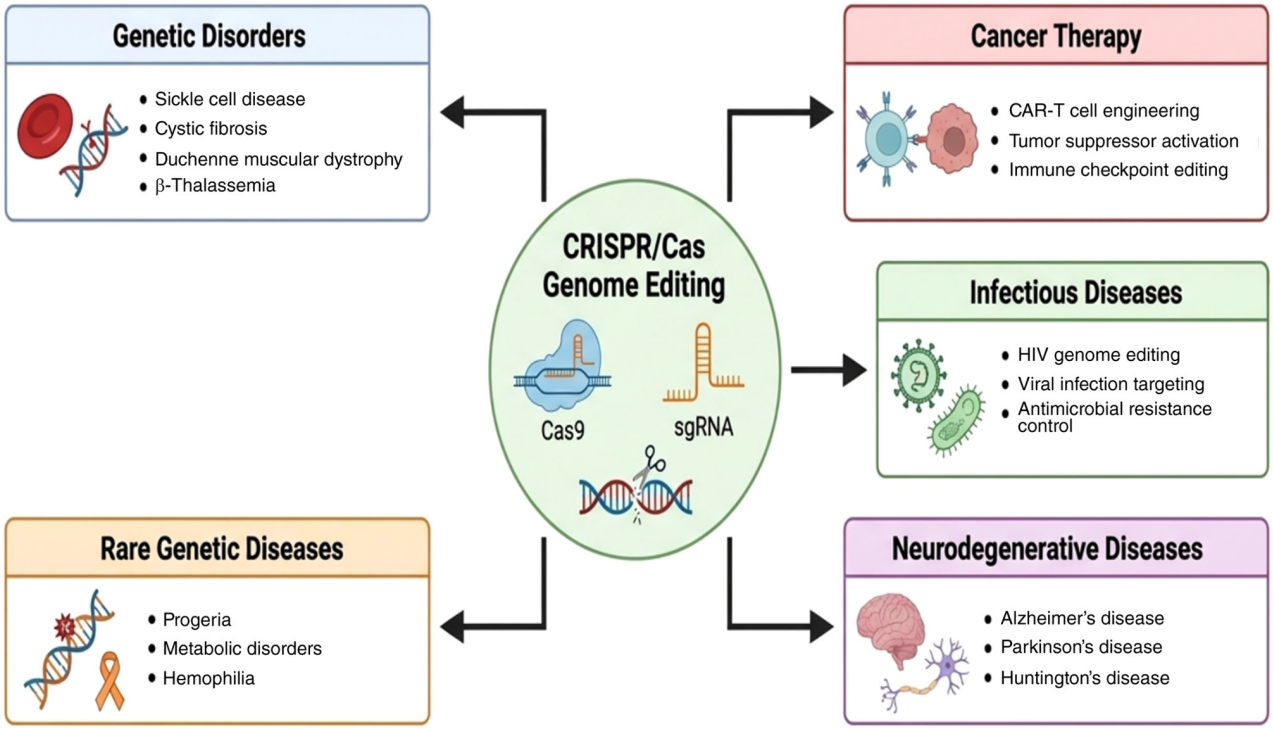


Figure 2. Therapeutic applications of CRISPR/Cas systems in human disease, including genetic disorders, cancer immunotherapy, infectious diseases and neurodegenerative disorders. Genome editing enables targeted modification of disease-associated genes and regulatory pathways, supporting the development of precision medicine approaches. CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; CAR, chimeric antigen receptor; sgRNA, single-guide RNA; HIV, human immunodeficiency virus.

CRISPR-based methods can introduce targeted mutations within a few months, accelerating experimental research. The ability to efficiently generate animal and cellular models has made CRISPR-Cas9 a tool in the study of human disease and the development of new therapeutic strategies (39) (Fig. 2).

4. CRISPR-Cas systems for the diagnosis of infectious disease

Infectious disease is a notable global health problem and requires rapid, sensitive and accurate diagnostic methods for effective clinical management and control of transmission. Conventional diagnostic methods, including PCR and serological assays, may require specialized equipment and have limitations in early-stage detection when pathogen levels are low (54). For this reason, nucleic acid-based detection methods serve an important role in infectious disease diagnosis. CRISPR-based diagnostic systems have emerged as useful tools for the detection of pathogen-derived nucleic acids. These methods rely on sequence-specific recognition of target DNA or RNA by CRISPR-associated enzymes guided by CRISPR RNA. Once the target sequence is recognized, cleavage of a reporter molecule generates a detectable signal. This principle has been applied in several diagnostic platforms for viral and bacterial pathogens (Table IV) (55).

CRISPR-based diagnosis of viral infection. One of the most widely studied applications of CRISPR diagnostics is the

Table IV. CRISPR (clustered regularly interspaced short palindromic repeats)-based diagnostic platforms for infectious disease.

Diagnostic platform	CRISPR enzyme	Target nucleic acid	Detection principle	Applications
SHERLOCK	Cas13	RNA	Guide RNA directs Cas13 to viral RNA; activated Cas13 cleaves reporter RNA producing a detectable signal	Detection of SARS-CoV-2, Zika and Dengue virus
DETECTR	Cas12	DNA	Cas12 recognizes target DNA and activates collateral cleavage of reporter DNA molecules	Detection of HPV and SARS-CoV-2
Cas9-based detection	Cas9	DNA	Cas9-guided cleavage combined with nucleic acid amplification and signal readout	Detection of bacterial genes or genetic mutations
CRISPR-microfluidic diagnostics	Cas12/ Cas13	DNA or RNA	Integration of CRISPR detection with microfluidic systems for rapid point-of-care testing	Portable pathogen detection systems

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; SHERLOCK, Specific High-sensitivity Enzymatic Reporter unLOCKing; DETECTR, DNA Endonuclease Targeted CRISPR Trans Repor.

detection of viral infection. Key platforms are DETECTR and SHERLOCK. DETECTR is based on the activity of Cas12, whereas SHERLOCK uses Cas13. These systems detect specific viral nucleic acid sequences with high sensitivity through CRISPR-guided target recognition followed by collateral cleavage of labeled reporter molecules (56).

In the DETECTR platform, gRNA directs Cas12 to a target DNA sequence. Following target recognition, Cas12 cleaves nearby single-stranded DNA reporters, producing a measurable signal. In the SHERLOCK platform, Cas13 is guided to target RNA and activation of Cas13 results in cleavage of nearby reporter RNA molecules. These systems have been investigated for the detection of several viral pathogens, including SARS-CoV-2, Zika and Dengue virus (57). Recent work has also explored the combination of CRISPR diagnostics with microfluidic devices to support portable and field-based testing (58). Such approaches may improve access to rapid diagnostic methods in low-resource settings (59).

CRISPR-based diagnosis of bacterial infection. CRISPR-based methods have been applied to the detection of bacterial pathogens. These approaches are relevant for the identification of antimicrobial-resistant bacteria, where rapid diagnosis is key for treatment decisions and infection control (60). CRISPR-based assays have been developed for the detection of bacterial species and resistance-associated genetic sequences (61,62). Cas12- and Cas13-based systems have been investigated for identifying pathogens in clinical samples such as blood, urine and cerebrospinal fluid (63). These diagnostic approaches demonstrate the sequence specificity and sensitivity of CRISPR systems, supporting their potential application in bacterial pathogen detection (60). Studies have reported CRISPR-based detection of bacterial pathogens such as *Salmonella, Listeria* and methicillin-resistant S. aureus, using gRNAs designed against conserved genomic regions (64,65)

Detection of nucleic acids using CRISPR systems. In addition to genome editing, CRISPR systems are adapted for nucleic acid detection. Their diagnostic use is based on the ability of CRISPR-associated proteins to recognize specific DNA or RNA sequences. Once activated, these enzymes generate a detectable readout through cleavage of reporter molecules. Type II CRISPR systems, including Cas9-based platforms, have been explored for molecular detection (59). One example is the Cas-exponential amplification reaction, which combines the sequence specificity of Cas9 with isothermal amplification and fluorescent readout (59,60). Such methods support sensitive detection of target nucleic acids without requiring complex instrumentation (65).

Advantages and limitations of CRISPR-based diagnostics. CRISPR-based diagnostic systems offer sequence-specific detection, rapid readout and the possibility of point-of-care application. In addition, they can be adapted to detect DNA or RNA targets, depending on the CRISPR enzyme used. At the same time, several limitations remain. Certain assays still require prior nucleic acid amplification and performance may vary depending on sample preparation, target abundance and assay design. Standardization, validation in clinical samples and large-scale implementation are needed before routine clinical use is established (66).

5. Functional genomics: CRISPR screening for target discovery

Genome-wide CRISPR screening is an important method for investigating gene function at large scale. These screening approaches allow researchers to identify genes involved in cell survival, proliferation, differentiation, drug resistance and other biological processes. In this method, cells are transduced with a library of sgRNAs, with each guide targeting a different gene. Following selection based on a defined phenotype, the

abundance of sgRNAs is measured by sequencing to determine which genes are associated with the observed biological response (67,68).

The CRISPR/Cas9 system derived from *S. pyogenes* is used in genome-wide screening; gRNA directs Cas9 to a specific genomic target through complementary base pairing (69). Cas9 introduces a DSB, which is repaired primarily through NHEJ, leading to insertions or deletions that disrupt gene function (70). In pooled screening experiments, a large population of cells receives a diverse sgRNA library (71). Following experimental selection, genomic DNA is isolated and the sgRNA regions are amplified and sequenced. Comparison of sgRNA abundance before and after selection allows identification of genes that influence the phenotype under study (72).

Genome-wide knockout screens. CRISPR/Cas9 knockout screening is used to identify genes essential for specific biological functions (73). This approach is used to study genes involved in tumor growth, resistance to therapy and synthetic lethality (67). Compared with RNA interference, CRISPR knockout screening provides more complete loss of gene function and improved target specificity (68). Genome-scale knockout libraries are delivered through lentiviral vectors, allowing stable expression of sgRNA in target cells (74).

CRISPR activation and interference screens. In addition to gene knockout, CRISPR systems can be used for transcriptional activation. In these approaches, catalytically inactive dead Cas9 (dCas9) is fused to transcriptional activation domains and guided to promoter regions by sgRNAs. This allows increased expression of selected genes without altering the DNA sequence itself. CRISPR activation screening has been used to identify genes associated with drug resistance, cell signaling and disease-related pathways (75). It has also been applied to study long non-coding RNAs and evaluate the functional effects of increased gene expression in cell models (76).

Target discovery in oncology via functional CRISPR screening. CRISPR/Cas9-mediated genome editing has revolutionized functional genomics, providing programmable tools to systematically interrogate the molecular mechanisms driving oncogenesis, tumor microenvironment evasion and acquired therapeutic resistance (77). High-throughput, genome-wide CRISPR knockout and activation/interference screens facilitate the parallel disruption or modulation of thousands of genes across pooled cell populations, enabling unbiased mapping of genetic dependencies essential for tumor cell survival and identification of novel druggable targets (78). Developed genome-scale CRISPR-Cas9 knockout libraries targeting 18,084 genes with 64,751 unique gRNAs in human cells. When used to elucidate melanoma resistance to the MAPK inhibitor vemurafenib, this framework mapped complex resistance networks (79). It successfully validated well-characterized tumor suppressors whose loss hyperactivates RAS-MAPK signaling, such as the neurofibromin regulator NF1 and the Mediator complex subunit MED12. Concurrently, it uncovered previously unrecognized therapeutic resistance hits, including the Hippo-pathway-linked tumor suppressor NF2, the ubiquitin ligase core component CUL3, and the chromatin-remodeling STAGA complex subunit TADA2A, all of which bypass BRAF inhibition through epigenetic or alternative transcriptomic rewiring (71).

CRISPR screening has defined synthetic lethal relationships in BRCA1/2-deficient tumors, identifying vulnerabilities such as poly(ADP-ribose) polymerase 1 ablation (80), enabling rational therapeutic combinations that selectively eliminate malignant clones. Pooled CRISPR screens have also been used to investigate immune checkpoint regulation, identifying regulators of major histocompatibility complex I antigen presentation, IFN-γ signaling (JAK1, STAT1) and immune-evasive pathways (81). While CRISPR screens have transformed target discovery, limitations remain. Screen results vary with cell line context, gRNA efficiency and selection conditions; findings typically fail to translate from 2D cultures to *in vivo* models (82). Clinical validation of screening-identified targets remains a bottleneck, with most hits failing to advance to therapeutic development. Despite these challenges, CRISPR functional genomics remains a dynamic platform for understanding tumor evolution, immune evasion and multi-drug resistance.

Data analysis: Model-based analysis of genome-wide CRISPR/Cas9 knockout and beyond. Analysis of genome-wide CRISPR screening data requires accurate identification of sgRNAs that are enriched or depleted following selection. Several computational tools have been developed for this purpose. One widely used method is model-based analysis of genome-wide CRISPR/Cas9 knockout, which evaluates changes in sgRNA representation and identifies candidate genes associated with the phenotype of interest (82,83). Such analytical tools are important for interpreting large-scale screening experiments and distinguishing meaningful biological signals from experimental noise.

6. Therapeutic applications: From *ex vivo* editing to *in vivo* gene correction

Gene therapy aims to correct, disrupt or replace disease-associated genetic sequences to treat human disorder. CRISPR-Cas systems provide a targeted method for modifying genomic DNA. The CRISPR-Cas9 system uses gRNA to direct the Cas9 nuclease to a specific DNA sequence, where a DSB is introduced. The break is repaired by cellular mechanisms, mainly NHEJ or HDR. These repair pathways make it possible to disrupt mutant genes or correct disease-causing variants (84). CRISPR-based gene therapy has been investigated in monogenic disorders, infectious disease and cancer (85,86). The technology has also been used in experimental systems such as induced pluripotent stem cells and animal models (87), which support the study of disease mechanisms and therapeutic development (88). Examples of clinical and preclinical applications are summarized in Table V.

Monogenic disease. Monogenic diseases are caused by mutations in a single gene and are considered suitable targets for genome editing approaches. Among these disorders, sickle cell disease and β-thalassemia are widely studied (89). Both conditions result from abnormalities in the β-globin gene or

Table V. Selected clinical and biomedical applications of clustered regularly interspaced short palindromic repeats -based genome editing in immunology and disease research.

Application	Target gene/ pathway	Disease	Research status	Translational challenges
Immune checkpoint editing	PD-1	Non-small cell lung cancer	Clinical trials using *ex vivo* edited T cells	Maintaining T cell metabolic fitness during extensive *in vitro* expansion and overcoming immunosuppressive mechanisms within the solid tumor microenvironment
Viral infection research	CCR5	HIV infection	Experimental and early clinical studies	Ensuring long-term hematopoietic engraftment safety and preventing viral escape through the emergence of resistant HIV variants
Autoimmune disease	FOXP3	Lupus and other immune disorders	Preclinical research	Risk of lineage instability and conversion of edited regulatory T cells into pathogenic effector phenotypes under chronic inflammatory conditions
Primary immunodeficiency	CD40L	X-linked hyper-IgM syndrome	Experimental gene-correction studies	Low homology-directed repair efficiency in quiescent hematopoietic progenitor cells and potential p53-mediated apoptosis following genome editing
Airway inflammation research	MUC18 (CD146)	Respiratory inflammation	Functional genomic research	Achieving efficient epithelial-specific delivery *in vivo* while minimizing systemic exposure and mucosal immune activation

CCCR5, C-C chemokine receptor type 5; CD40L, CD40 ligand; MUC18, mucin 18.

its regulation and lead to severe hematological complications. CRISPR-based therapeutic approaches for these disorders primarily focus on correction of the disease-causing mutation or reactivation of fetal hemoglobin expression (90). Clinical study have shown encouraging outcomes in patients with sickle cell disease and β-thalassemia, supporting the use of genome editing as a therapeutic strategy in hematological disorders (91). CRISPR has also been investigated in other inherited disorders, including cystic fibrosis and hereditary tyrosinemia. In experimental hereditary tyrosinemia, correction of the fumarylacetoacetate hydrolase mutation has been reported following *in vivo* delivery of CRISPR/Cas9 components to the liver (92). These studies indicate that CRISPR-based approaches may be useful for the treatment of monogenic disease (93).

Cancer immunotherapy. CRISPR/Cas9 genome editing is being actively investigated as a potential strategy for the targeted treatment of cancer, offering a programmable platform that allows precise modification of key genes involved in tumor growth and immune regulation. Clinical applications comprise two main approaches: *Ex vivo* PD-1 disruption in autologous T cells and CRISPR-engineered chimeric antigen receptor (CAR)-T cell therapies (94).

Clinical trials for lung cancer using CRISPR-based gene therapy. Studies have explored the clinical utility of CRISPR-mediated gene editing (95), to amplify host anti-tumor immune responses within the clinical setting (96). The first reported clinical study applying CRISPR-Cas9 gene editing in patients with lung cancer was conducted in China (97): Patient T cells were edited *ex vivo* to permanently disrupt the expression of PD-1 gene, an immune checkpoint that suppresses T cell activity upon engagement with its ligands in the tumor microenvironment. The edited, checkpoint-ablated T cells were expanded *in vitro* and reinfused back into patients presenting with advanced, refractory non-small cell lung cancer (NSCLC) (98). PD-1 disruption could successfully enhance T cell anti-tumor activity and promoted durable responses in a subset of treated individuals. However, the study also highlighted clinical limitations that require active bioengineering solutions, including variable *ex vivo* editing efficiencies between patient samples, potential immune reactions against the edited cellular products and the mandatory requirement to evaluate potential off-target effects. Driven by these early insights, clinical research in oncology continues to expand, with engineered T cell therapies approaching tumor response rates up to 85% in select cohorts (99). Clinical trials are currently evaluating CRISPR-modified immune cells to treat both LC and alternative hyper-mutated solid tumors, where clinical efficacy for NSCLC demonstrates a reported 38% positive outcome rate across early-phase trial dynamics (100-102) (Fig. 3). CRISPR-edited T cells are strategically combined with immune checkpoint inhibitors, such as pembrolizumab, to improve therapeutic response rates and overcome tumor-mediated immunosuppression (103). A prominent example is trial NCT02793856, using PD-1 knockout engineered T cells for metastatic NSCLC (104,105).

CRISPR-engineered CAR-T cells. CRISPR-Cas technology has advanced oncology, particularly through the genetic

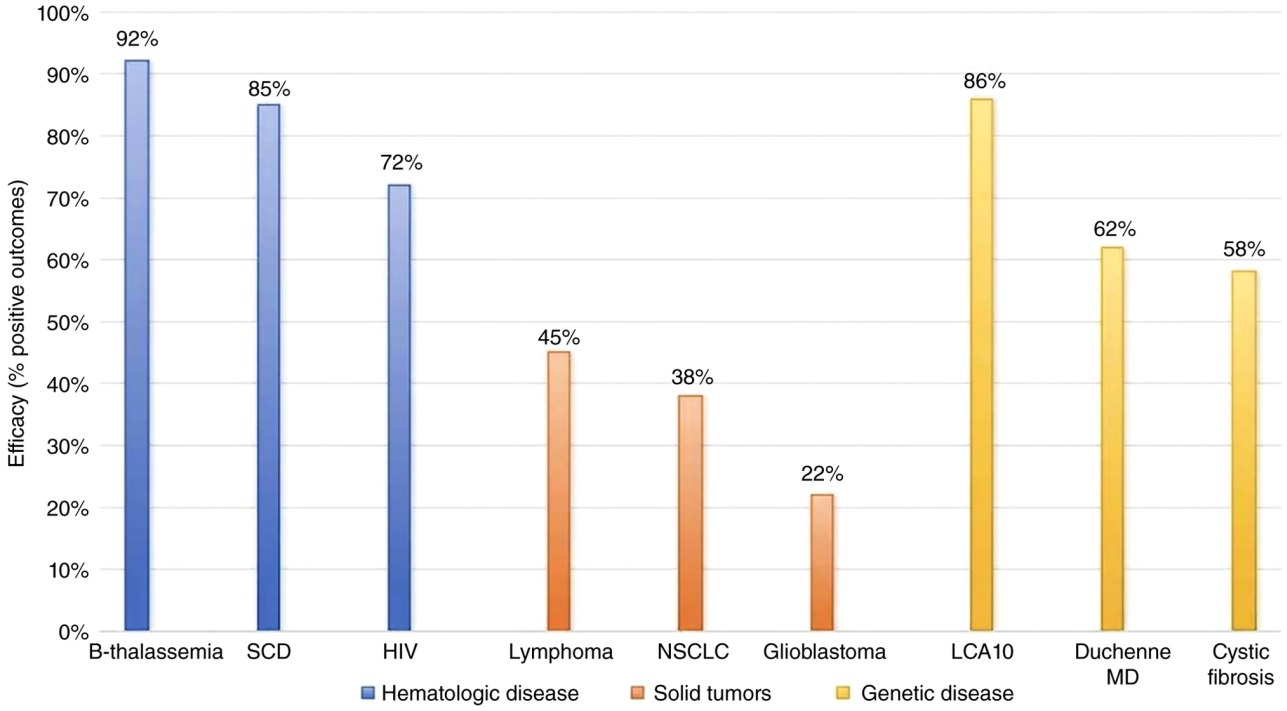


Figure 3. Reported efficacy-related outcomes and therapeutic benchmarks of CRISPR-based approaches across disease categories. The values were compiled from published clinical trials, preclinical experimental studies, and translational research reports. Values represent disease-specific therapeutic outcomes, editing efficiencies, or reported translational benchmarks and should not be interpreted as directly comparable clinical efficacy rates across disease categories. β-thalassemia, beta-thalassemia; SCD, sickle cell disease; HIV, human immunodeficiency virus; NSCLC, non-small-cell lung cancer; LCA10, Leber congenital amaurosis 10; MD, muscular dystrophy (74-84 and 95).

engineering of adoptive cell transfer therapies. The primary clinical application is the optimization of CAR-T therapy (87). *Ex vivo* genome editing enables the removal of inhibitory signaling pathways and the disruption of endogenous T-cell receptors to reduce the risk of graft-vs.-host disease (GvHD) in allogeneic adoptive cell therapies (104,106). Targeted disruption of genes encoding co-inhibitory immune checkpoint receptors, including programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4, has been investigated to improve the antitumor activity of engineered T cells (107,108). By reducing inhibitory signaling, genetic editing may enhance T-cell functionality and sustain effector responses during chronic antigen exposure, although the effects depend on the specific target and editing strategy (109,110). Early clinical studies of CRISPR-modified CAR-T cells in hematological malignancies have demonstrated promising antitumor activity, with a phase I study of CRISPR-engineered CAR-T cells reporting an 87.5% complete remission rate in a small cohort of patients with relapsed or refractory B-cell non-Hodgkin lymphoma (111,112). The clinical pipeline encompasses autologous and universal allogeneic CAR-T-cell therapies, as well as genome-editing approaches involving hematopoietic stem cells and other therapeutic cell types (113,114). Multiplex gene editing can disrupt endogenous T-cell receptor genes and co-inhibitory immune-checkpoint genes, while targeted integration of CAR transgenes at defined genomic loci, such as the T-cell receptor alpha constant (TRAC) locus, can improve control over CAR expression (115). Electroporation-based delivery of CRISPR/Cas9 components has enabled efficient genome editing in engineered T cells; however, genomic safety, off-target effects, and preservation of cell potency require careful evaluation (116).

Barriers to solid tumor immunotherapy. Despite early clinical successes, NSCLC remains difficult to treat because of tumor heterogeneity, poor delivery and immune escape. This translational gap is primarily due to three biological barriers. Effective editing of solid tumors requires delivery vehicles to cross vascular barriers and penetrate dense, fibrotic extracellular matrices. Certain non-viral delivery platforms preferentially accumulate in the liver, leaving solid tumor cells insufficiently edited (117,118) Tumor populations are genetically diverse and continuously evolving. Editing a single oncogenic driver or surface antigen may fail to reach all malignant clones, allowing unedited cells to escape immune detection and drive relapse (119). Malignant cells typically exhibit pre-existing genomic instability and compromised DNA repair mechanisms, including TP53 deficiency. Sustained Cas9 activity or multiple DSBs induce chromosomal translocations or large-scale structural rearrangements. This may inadvertently select for aggressive p53-deficient clones, raising long-term safety concerns (120). Although early clinical study demonstrate the feasibility of CRISPR-engineered cell therapies, achieving durable responses in solid tumors remains a challenge (121). These biological complexities must be addressed to improve the safety and efficacy of CRISPR-based interventions in oncology (95).

Current status of CRISPR/Cas in allergy, immunology and autoimmune disorder. CRISPR-based genome editing has

Table VI. Clustered regularly interspaced short palindromic repeats applications in gene therapy.

Disease	Study stage	Outcome	Therapeutic approach
Sickle cell disease	Clinical	Increased hemoglobin production and decreased disease manifestation	Editing of hematopoietic stem cells
β-thalassemia	Clinical	Decreased transfusion requirement	Editing of hematopoietic stem cells
Leber congenital amaurosis	Clinical	Improvement in visual function in treated individuals	*In vivo* retinal editing
Hereditary transthyretin amyloidosis	Clinical	Decreased circulating transthyretin levels	*In vivo* liver-directed editing
HIV	Early clinical/ experimental	Decreased viral persistence or susceptibility	Editing of proviral DNA or host entry factors
Cancer (CAR-T cell therapy)	Early clinical	Improved anti-tumor immune activity	Editing of immune checkpoint-associated genes
Duchenne muscular dystrophy	Preclinical	Partial restoration of dystrophin expression	CRISPR-Cas9-mediated deletion of selected exons to restore the reading frame and potentially recover dystrophin expression'

HIV, human immunodeficiency virus; CAR, chimeric antigen receptor.

transformed the landscape of molecular and translational immunology by delivering a programmable, site-specific platform to systematically interrogate and modulate the genetic circuitry governing immune cell differentiation lineages, host receptor signaling cascades and chronic inflammatory response pathways. These innovations have provided mechanistic insights into the underlying etiology of systemic autoimmune disorder, persistent viral reservoirs and primary immunodeficiency, introducing actionable therapeutic axes that bypass the clinical limitations of conventional, non-specific small-molecule immunosuppressants (122).

A contested frontier in this domain involves the deployment of CRISPR platforms to achieve functional cures against chronic viral pathogens that establish long-term persistence within the host genome. Translational strategies either directly target integrated viral DNA templates or permanently ablate non-redundant host cell surface co-receptors essential for viral entry kinetics (123). A clinical paradigm of this approach is the targeted disruption of the CCR5 chemokine receptor in patient-derived autologous hematopoietic stem and progenitor or mature ($CD4^+$) T cells, which has been extensively evaluated to restrict human immunodeficiency virus (HIV)-1 viral entry and control systemic viral replication without requiring lifelong antiretroviral therapy (124). Concurrently, functional genome editing is a key toolkit in autoimmune disease research, where the therapeutic goal has shifted from systemic immune suppression to the precise restoration of peripheral self-tolerance. In complex autoimmune experimental systems, precise CRISPR-mediated gene modulation is used to correct, reactivate or tune key master transcription factors that dictate regulatory T cell (Treg) lineage specification and suppressive function, most notably the FOXP3 gene axis (125). CRISPR-mediated stabilization of antigen-specific Tregs) offers a powerful approach to restore immune homeostasis. This strategy can precisely suppress the overactive T and B-cell responses that drive chronic autoimmune pathologies, including systemic lupus erythematosus and rheumatoid arthritis (126) (Fig. 3; Tables V and VI).

Infectious disease

HIV. CRISPR-Cas systems have been explored as therapeutic tools against infectious diseases, particularly viral infection. In these applications, genome editing may target either the viral genome or host factors required for viral entry and replication (127). One important example is HIV, which integrates its genome into host cells and is difficult to eliminate completely with conventional antiretroviral therapy (128). CRISPR-based strategies have been investigated to disrupt the integrated HIV provirus or to modify host receptors such as CCR5, which is required for viral entry into certain immune cells. These approaches are being studied as possible methods for long-term viral control (129). The use of CRISPR technology in infectious disease therapy remains largely experimental, and several issues require attention, including delivery efficiency, editing specificity and long-term safety (130).

Alternative gene-editing strategies against HBV and HCV. Chronic infection with HBV and HCV remains an important clinical problem. For HBV, one major challenge is the persistence of covalently closed circular DNA (cccDNA) in infected hepatocytes (131). This episomal DNA serves as a stable template for viral replication and is not fully eliminated by conventional antiviral therapy. CRISPR/Cas9 has been investigated as a potential method to disrupt HBV cccDNA and reduce viral replication (132). By contrast, HCV is an RNA virus that replicates in the cytoplasm, making RNA-targeting systems such as Cas13 of interest. These approaches differ

Table VII. Genome-editing platforms used in therapeutic research.

Parameter	CRISPR/Cas9	ZFNs	TALENs	Prime editing
Targeting design	Guide RNA-based	Protein-based	Protein-based	Guide RNA-based
Editing flexibility	High	Moderate	Moderate	High
Multiplex editing	Possible	Limited	Limited	Possible
Off-target concern	Present	Present	Present	Avoids conventional double-strand breaks, although unintended edits and by-products may occur
Current therapeutic use	Broad experimental and clinical use	Limited clinical use	Limited clinical use	Early-stage development

CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; ZFN, zinc finger nuclease; TALEN, transcription activator-like effector nuclease.

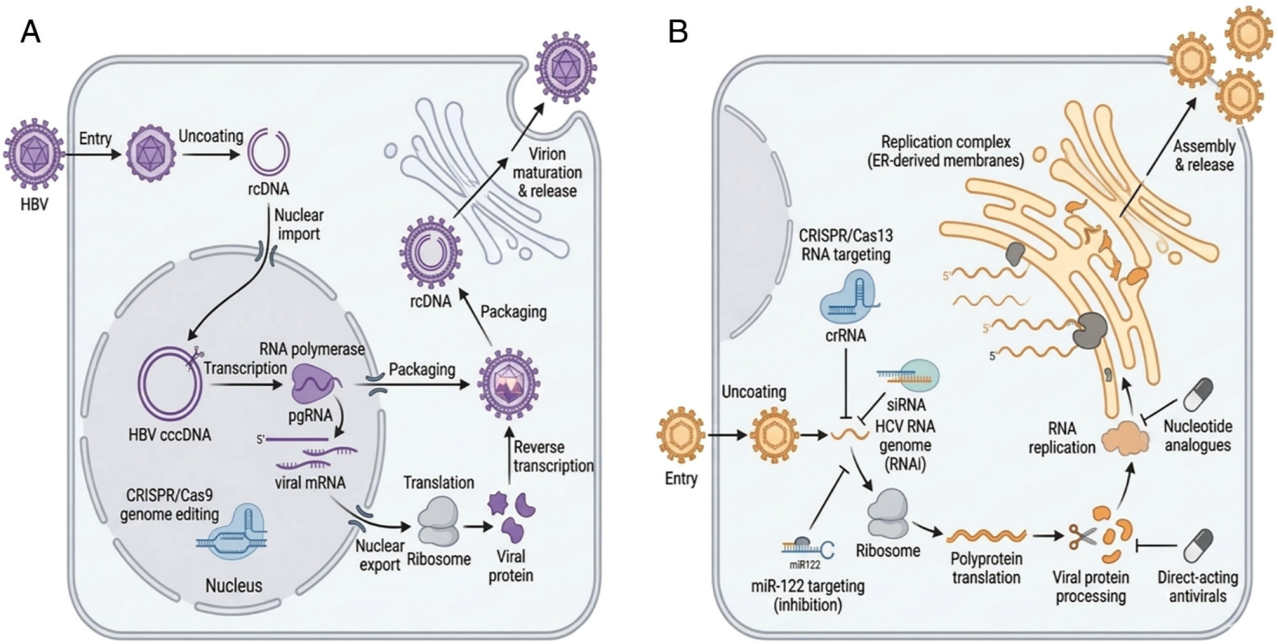


Figure 4. Schematic representation of HBV and HCV replication pathways relevant to gene-editing strategies. (A) HBV replication involves the formation of cccDNA in the host cell nucleus, which serves as a transcriptional template for viral RNA and drives persistent infection. CRISPR-Cas9 tools have been investigated to targetedly disrupt this episomal reservoir. (B) HCV replicates exclusively within the cytoplasm via an RNA intermediate without forming a nuclear DNA intermediate. Consequently, RNA-targeting mechanisms, including Cas13-based platforms, are uniquely suited for targeting the HCV genome. HBV, hepatitis B virus; HCV, hepatitis C virus; cccDNA, covalently closed circular DNA; CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; rcDNA, relaxed circular DNA; pgRNA, pregenomic RNA; ER, endoplasmic reticulum; crRNA, CRISPR RNA; siRNA, small interfering RNA; RNAi, RNA interference; miR, microRNA.

from DNA editing strategies used for HBV and reflect the distinct biology of the two viruses. Other nucleic acid-based approaches, including RNA interference, have been tested for HBV and HCV (133). CRISPR-based approaches offer the advantage of directly targeting and destroying persistent viral genetic material within host cells (134). The replication cycles and structural variations of HBV and HCV, along with their respective gene-editing vulnerabilities, are summarized in Fig. 4 and Table VII. This data is adapted from published comparative analyses of genome-editing platforms, including CRISPR-Cas9, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and prime editing technologies (135).

Comparative feasibility and clinical maturity of CRISPR-Cas modalities. To evaluate the translational value of genomic medicines, a distinction must be established between molecular diagnostics, *ex vivo* cell replacement therapies and *in vivo* direct gene correction, based on their relative clinical maturity and implementation proximity. The clinical maturity, delivery characteristics and therapeutic targets of CRISPR-Cas applications across different developmental phases are summarized in Table VIII.

Phase I: Established ex vivo cell modalities. Ex vivo applications currently hold the most dominant position in clinical therapeutic development because editing cells outside the body

Table VIII. Clinical maturity, delivery characteristics, and therapeutic targets of clustered regularly interspaced short palindromic repeats-based applications across developmental phases.

Modality	Clinical maturity	Biological vector profile	Primary target/identifier	(Refs.)
Molecular diagnostics	Near-term/highly scalable	Amplification-free platforms utilizing Cas12/Cas13	Pathogen detection; 53% infectious disease market dominance	(47)
Phase I: Mature *ex vivo* therapies	Established clinical footprint; regulatory precedents active across hematological pipelines	High-efficiency electroporation or viral transduction executed *ex vivo*; no systemic delivery	Transfusion-dependent β-thalassemia and severe sickle cell disease	(100,101)
Phase II: Tissue-isolated *in vivo* therapeutics	Emerging *in vivo* modality; partially evades host immunity due to anatomical isolation	Localized retinal injection; local administration leverages immune-privileged status	CEP290 gene correction for Leber congenital amaurosis 10 blindness	NCT03872479 (102)
Phase III: Systemic *in vivo* direct editing platforms	Experimental frontier; restricted by biological barriers and systemic toxicity	Systemic intravenous delivery; risk of pre-existing host humoral and cell immune responses	Transthyretin knockout in liver tissue; PCSK9 adenine base editing in primate models	NCT04601051 (103)

Cas, clustered regularly interspaced short palindromic repeats-associated protein; CEP290, centrosomal protein 290; PCSK9, proprotein convertase subtilisin/kexin type 9.

bypasses systemic anatomical barriers and enables strict, high-throughput quality control before transplantation (136,137). Prominent examples approaching routine implementation include the Phase 1/2 trials NCT03655678 (138) and NCT03745287 (139) (evaluating BCL11A target editing in $CD34^+$ human hematopoietic stem and progenitor cells for transfusion-dependent β-thalassemia and severe sickle cell disease) and NCT02793856 (140) (using PD-1 knockout engineered T cells for metastatic NSCLC). The clinical success of *ex vivo* CRISPR-based hematopoietic stem cell editing has further been demonstrated by the development of approved genome-edited therapies such as exagamglogene autotemcel for severe sickle cell disease, highlighting the current translational maturity of controlled cellular engineering approaches (141).

Phase II: Emerging, localized in vivo therapeutics. Direct *in vivo* gene editing is a clinically viable approach when targeted locally via site-specific injections into highly isolated, specialized target organs. Local tissue injection enables precise editing while avoiding systemic clearance mechanisms, as demonstrated by the localized delivery of AAV5 viral vectors to correct mutations in the centrosomal protein 290) gene for Leber congenital amaurosis 10 blindness (NCT03872479) (126). Because the eye is an anatomically immune-privileged compartment, this approach decreases the risk of systemic host immune responses or off-target genotoxicity in bystander cells, establishing localized *in vivo* delivery as the clinical frontrunner for structural organ editing (129).

Phase III: Complex systemic in vivo gene correction. The least mature yet most broad-reaching modality involves systemic, intravenous administration designed to treat multi-systemic disorders or genetic diseases that affect internal organs inaccessible by local injection. Systemic *in vivo* engineering has achieved validation in hepatic targets due to the natural tendency of synthetic nanoparticles (NPs) to accumulate in liver tissue. The first-in-human systemic trial (NCT04601051) demonstrated the feasibility of using lipid nanoparticles (LNPs) to deliver transient Cas9 mRNA to permanently knock out transthyretin in patients with hereditary transthyretin amyloidosis, balancing initial therapeutic efficiency with systemic exposure (142,143).

Similarly, systemic LNP-mediated delivery of adenine base editors targeting the regulatory gene PCSK9 (proprotein convertase subtilisin/kexin type 9) in non-human primates has shown robust, long-term therapeutic benefit (144). Because PCSK9 normally binds to hepatic low-density lipoprotein receptors (LDLR) and targets them for lysosomal degradation, base-editor-mediated knockout of PCSK9 prevents receptor destruction. This allows continuous recycling of LDLR to the hepatocyte surface, lowering serum LDL cholesterol over several months without causing double-strand DNA breaks (DSBs) or long-term immune complications (145). Recent advances in nucleic acid-based therapeutics further demonstrate the expanding potential of transient genome modulation strategies while highlighting remaining delivery and safety limitations (146). However, achieving comparable success in complex extrahepatic solid malignancy or hidden viral reservoirs (such as integrated HIV-1 or latent viral targets) remains experimental. These applications are hindered by an inability to target deep extrahepatic tissue without causing widespread off-target exposure or triggering acute host immune countermeasures (147).

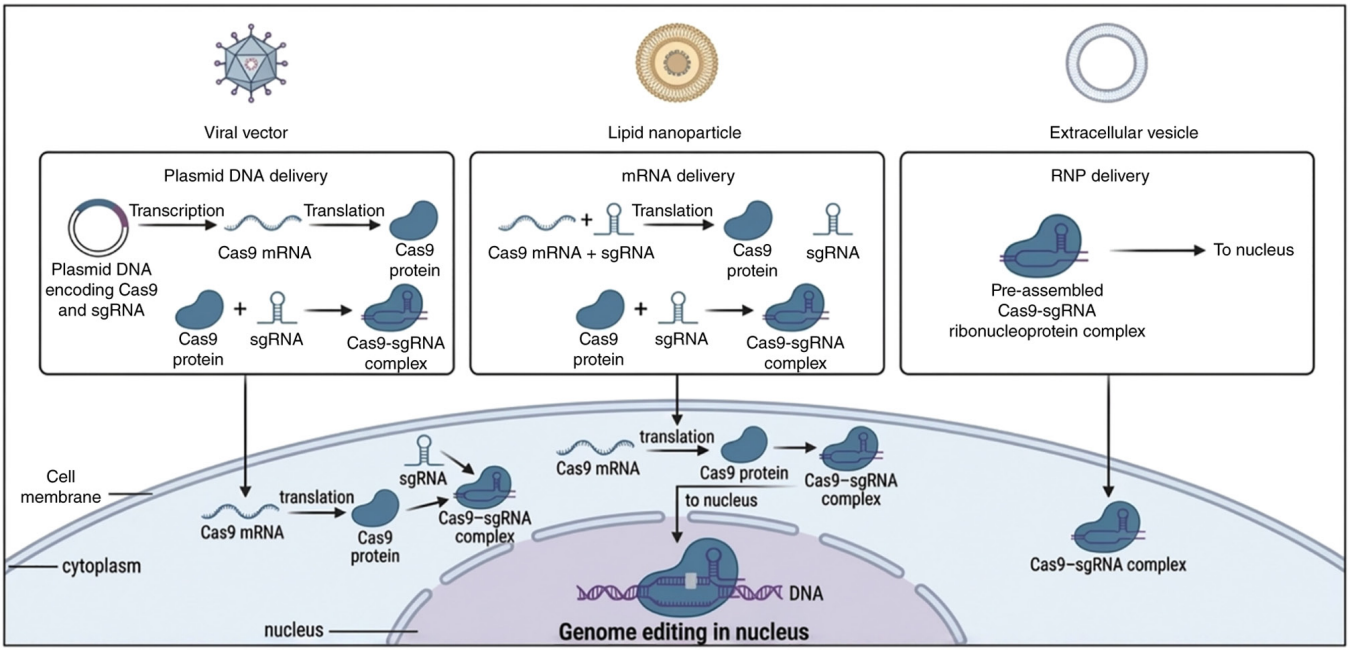


Figure 5. Delivery strategies for clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 components. Cas9 can be delivered into cells in several forms, including plasmid DNA encoding Cas9 and guide RNA (gRNA), mRNA, or pre-assembled Cas9 ribonucleoprotein (RNP) complexes. Delivery may occur through viral vectors, lipid nanoparticles, or extracellular vesicles. These approaches aim to transport Cas9 and gRNA into the cell and nucleus where genome editing can occur. RNP, ribonucleoprotein; Cas, CRISPR-associated protein; sgRNA, single-guide RNA.

Delivery strategies for CRISPR components. Efficient delivery of these CRISPR components into specific target cells remains an important consideration for all clinical applications. Delivery approaches have been explored across preclinical and clinical pipelines, including engineered viral vectors, LNPs and pre-assembled RNP complexes (Fig. 2). Viral delivery vectors, such as lentiviruses and AAVs, provide high baseline cell entry efficiencies but carry notable regulatory risks related to persistent host immune responses or low-frequency insertional mutagenesis (148). Conversely, non-viral delivery methods, including LNPs which currently achieve ~58% hepatocyte uptake efficiency in clinical settings, and cell-derived extracellular vesicles, are being investigated as alternative, safer strategies for delivering transient CRISPR components to target cell populations while minimizing off-target kinetics (149) (Fig. 5).

7. Challenges, limitations and unresolved issues in CRISPR-Cas application

The application of CRISPR-Cas systems in research and therapy has progressed but limitations remain regarding the safety, efficiency, reproducibility and clinical translation of genome-editing approaches. Challenges include technical barriers, biological variability, regulatory concerns and incomplete long-term clinical data.

Editing efficiency and delivery challenges. Efficient and safe delivery of CRISPR components to target cells remains a challenge for therapeutic genome editing, particularly for tissues such as the brain, skeletal muscle and heart, which are difficult to reach due to biological barriers such as the blood-brain barrier (BBB) and dense extracellular matrices of myofibers. Even highly active nucleases or base editors cannot achieve therapeutic benefit without effective cell delivery. Among technical challenges that limit clinical translation, inefficient delivery to target tissues remains one of the most notable barriers, affecting editing efficiency and raising concerns regarding safety, reproducibility and long-term therapeutic outcomes (149).

Successful genome editing requires the safe and effective transport of Cas nucleases, gRNAs or RNP complexes into specific tissue while minimizing toxicity and unintended biodistribution (76). Viral vectors, particularly AAVs, have demonstrated high delivery efficiency and prolonged expression, however, they are constrained by limited cargo capacity, potential immunogenicity and the risk of insertional mutagenesis (150). By contrast, non-viral delivery systems such as LNPs, extracellular vesicles and polymer-based carriers offer improved safety profiles but often exhibit lower transfection efficiency and decreased tissue specificity (151).

Viral delivery systems. AAVs have traditionally served as the gold standard for *in vivo* delivery due to their robust transduction efficiency across multiple cell types. However, high-dose systemic administration typically triggers innate and adaptive immune responses, causing adverse events such as hepatotoxicity and thrombotic microangiopathy (152). Pre-existing neutralizing antibodies limit patient eligibility. Although AAVs largely remain episomal, low-frequency insertional mutagenesis is a regulatory concern. Constitutive long-term expression of Cas9 delivered via viral DNA also extends the window for off-target cleavage and may stimulate T cell-mediated immune responses.

Lentiviral vectors allow larger cargo capacity and are efficient for *ex vivo* editing, such as in T or hematopoietic stem cells, but carry risks of insertional mutagenesis and persistent

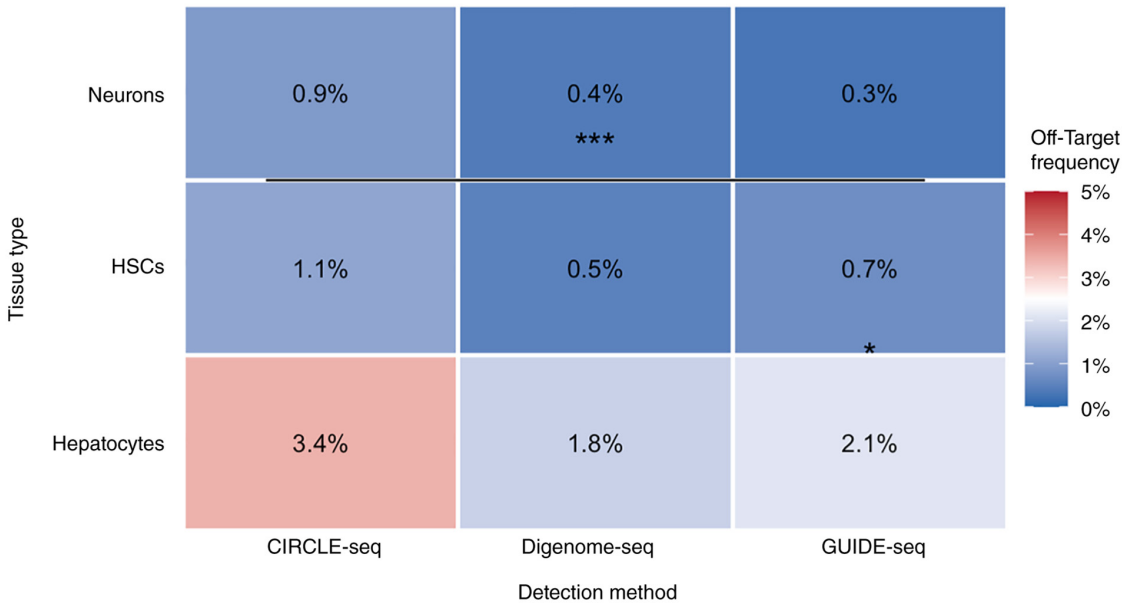


Figure 6. Off-target detection methods across tissue types. Off-target editing frequencies detected by CIRCLE-seq, Digenome-seq, and GUIDE-seq across neurons, hematopoietic stem cells (HSCs), and hepatocytes [adapted from reference 134]. Detection rates vary significantly by method and tissue architecture, with hepatocytes demonstrating the highest off-target cleavage frequencies across all evaluation platforms. Statistical significance indicators reflect variations relative to baseline controls: *P<0.05, and *** indicates P<0.001. HSC, hematopoietic stem cell; CIRCLE-seq, circularization for *in vitro* reporting of cleavage effects by sequencing; Digenome-seq, digested genome sequencing; GUIDE-seq, genome-wide unbiased identification of DSBs enabled by sequencing.

nuclease expression. Adenoviral vectors, which do not integrate, decrease insertional risk but exhibit higher immunogenicity, limiting repeated administration (153).

Non-viral delivery systems. Non-viral platforms, particularly LNPs, provide superior safety by enabling transient delivery of mRNA or RNP complexes. Transient expression minimizes genomic integration and limits off-target activity. However, standard LNP formulations exhibit hepatic tropism due to apolipoprotein E (ApoE) opsonization, resulting in low efficiency in extrahepatic tissues such as the brain and skeletal muscle, where delivery remains prohibitively low (149).

Delivery barriers and pre-existing immunity. Inefficient tissue-specific delivery and host immunogenicity hinder the clinical translation of CRISPR-based therapeutics. Standard non-viral carriers exhibit passive tropism, with conventional ionizable LNPs distributing predominantly to hepatocytes (>80% accumulation) via ApoE opsonization. This liver-capture barrier limits the entry efficiency into non-hepatic somatic tissue such as skeletal muscle, lung epithelium or the central nervous system where BBB penetrance is <1% (154).

Compounding this physical entrapment is the challenge of pre-existing human adaptive immunity against microbial effector nucleases. Epidemiological data confirm that a notable portion of the human population possesses anti-Cas9 memory pools, with pre-existing antibody prevalence as high as 58% for SpCas9 and higher reactive rates (78%) against SaCas9 (155). Systemic administration of viral vectors containing these bacterial effectors triggers memory $CD8^{+}$ T lymphocyte activation, which completely eliminates and destroys gene-edited cell populations within 12 weeks of exposure, causing safety concerns and eliminating the possibility of required repeat dosing (156).

Off-target effects and genome integrity. Despite advances in CRISPR-Cas technology, off-target genome editing is a barriers to its widespread clinical application. Off-target effects occur when the gRNA directs Cas nucleases to genomic regions that share partial sequence similarity with the target, resulting in unintended DNA cleavage and mutagenesis. Such unintended modifications may generate insertions or deletions (indels), chromosomal rearrangements, activation of oncogenes or disruption of tumour suppressor genes, thereby raising concerns regarding genomic instability and potential malignant transformation (157).

The frequency of off-target events varies according to gRNA design, target sequence composition, cell type, chromatin accessibility and the detection methods employed (Fig. 6). Although improvements have been achieved in recent years, accurate assessment of off-target activity *in vivo* is challenging, particularly in clinical settings where direct genome-wide monitoring is difficult (158). Consequently, long-term safety concerns remain regarding the persistence of unintended genetic alterations in edited tissue (159).

Several technological strategies have been developed to minimize off-target editing. High-fidelity Cas9 variants, including SpCas9- SpCas9-HF1 (Streptococcus pyogenes Cas9 high-fidelity variant 1) and evolved (evo)Cas9, HiFi-Cas9, and Cas9-R63A/Q768A (147), have demonstrated improved specificity while maintaining therapeutic editing efficiency. These engineered nucleases decrease non-specific interactions between Cas9 and genomic DNA, thereby increasing dependence on precise gRNA-DNA pairing. In parallel, advances in gRNA optimization, including truncated gRNAs, improved seed sequence selection and machine-learning-based design platforms such as sgDesigner (160), DeepCRISPR (161) and CRISPR Design (162), have enhanced editing precision.

Challenges in large-fragment genome editing and precision knock-in engineering. Although CRISPR-Cas systems efficiently generate gene knockouts and small sequence modifications, precise insertion, replacement or correction of large DNA fragments remains technically challenging. Most large-fragment editing strategies depend on HDR, which generally exhibits low efficiency, particularly in non-dividing or slowly dividing cells. The predominance of error-prone NHEJ pathways limits precise genome correction and may result in unintended genetic alterations (163).

Base and prime editing, CRISPR-associated transposases and HDR-enhancing strategies have shown promise in improving precision repair (164). However, achieving consistent and clinically relevant editing efficiencies for large genomic modifications remains a challenge (165). Further optimization of repair pathways, editing tools and delivery technologies will be required before large-scale genome correction can be routinely applied in clinical settings (166).

Another obstacle in large-fragment genome editing is the limited cargo capacity of current delivery vectors. AAV, a delivery platforms for *in vivo* genome editing, can package ~4.7 kb of therapeutic genetic material (167). Certain advanced genome-editing systems, including base and prime editors, exceed this capacity, necessitating dual-AAV delivery strategies (14,168). Although dual-vector approaches have demonstrated therapeutic editing in the liver, central nervous system, skeletal muscle and cardiac tissue, editing efficiencies remain variable and often lower than those achieved with smaller editing constructs (136). These limitations highlight the need for improved delivery systems capable of accommodating large editing cargos while maintaining high editing efficiency and safety (169).

The clinical translation of genome editing has advanced through target disruption via error-prone NHEJ. However, executing site-specific precision knock-in therapy remains constrained by the biological inefficiency of HDR (165). HDR is restricted to the late S and G2 phases of the cell cycle, rendering this pathway virtually quiescent in post-mitotic or slowly dividing somatic cells, such as mature neurons, hepatocytes and cardiomyocytes (170). When a DSB is introduced, the host cell endogenous repair machinery exhibits a kinetic preference for the blunt-end ligation pathways of NHEJ over template-dependent HDR (171). This competitive imbalance results in baseline precise integration rates <1-5% in target tissues, with the remaining events dominated by unexpected indels at the cut site (172).

This low baseline efficiency becomes progressively worse as the physical size of the donor template increases, introducing hurdles for large DNA insertion and macroscopic gene replacement. Shuttling larger genetic payloads (>1 kb) requires complex donor architectures such as double-stranded DNA (dsDNA) plasmids, long single-stranded DNA or AAV genomes which face steep intracellular delivery barriers (173). Large dsDNA templates trigger independent innate immune sensing networks via cytosolic cGAS/STING pathways, inducing high cellular toxicity and cell death before integration. Long single-stranded DNA donors avoid immune sensing but suffer from rapid intracellular degradation by endogenous nucleases. Additionally, supplying these large templates via viral vectors introduces a physical packaging capacity threshold of ~4.7 kb, making the simultaneous delivery of long corrective templates and heavy Cas9 machinery in a single vehicle challenging (174).

Even when a large donor template reaches the nucleus, the biophysical kinetics of the strand-invasion process decrease with increasing cargo size. For multi-kilobase gene replacements such as inserting full-length dystrophin or cystic Fibrosis Transmembrane Conductance Regulator) coding sequences, the efficiency of accurate homologous recombination is near-zero in clinical somatic targets (175,176). nstead of precise integration, large linear donor DNA fragments may undergo microhomology-mediated end joining (MMEJ) or random integration, potentially resulting in partial insertions, tandem arrays of donor DNA copies (head-to-tail concatemers), or unintended genomic insertions. These events may disrupt endogenous genes or regulatory elements, alter gene expression, and potentially contribute to genomic instability or abnormal clonal expansion, raising concerns about the genomic integrity and therapeutic safety of the corrected cell product (154).

To overcome limitations associated with conventional homology-directed repair (HDR)-dependent knock-in, several emerging genome-engineering strategies aim to facilitate large DNA insertions without introducing programmed DNA double-strand breaks (DSBs). These include twin prime editing (twinPE), which uses two prime-editing guide RNAs (pegRNAs) to generate complementary DNA flaps and enable targeted sequence replacement or the installation of recombinase recognition sites, and prime-editing-assisted site-specific integrase gene editing (PASSIGE), which combines prime editing with site-specific recombinases to integrate large DNA cassettes (177,178). Bxb1 integrase, a serine recombinase derived from bacteriophage, catalyzes directional recombination between compatible attachment sites, conventionally designated attB and attP. Evolved variants, including evoBxb1 and eeBxb1, have been engineered to improve recombination-mediated integration efficiency (179). In PASSIGE-based systems, prime editing installs an attachment site at the desired genomic locus, after which the integrase mediates insertion of a donor DNA cassette. In human cell lines, evolved Bxb1-based systems have achieved integration efficiencies of approximately 20-46% across tested loci, with efficiencies of up to 30% reported in primary human fibroblasts. CRISPR-associated transposases (CASTs) represent a complementary approach, using RNA-guided DNA targeting coupled to transposition machinery to insert DNA without relying on conventional HDR; engineered evoCAST systems have achieved 10-25% integration efficiencies for kilobase-sized DNA cargoes across tested human genomic sites (180). Although these technologies may reduce reliance on DSB-dependent repair and cell proliferation, their efficiency, insertion accuracy, off-target activity, delivery, and long-term safety remain dependent on the specific platform and target. Further validation is therefore required before they can be considered clinically established gene-replacement strategies.

Biological constraints: Immunological barriers to bacterial effectors. After cellular delivery has been achieved, a second major safety consideration is the preservation of genomic integrity and the long-term stability of edited cells.

Cas9-induced DNA double-strand breaks (DSBs) can activate the p53-mediated DNA damage response, leading to cell-cycle arrest, reduced cell viability, or apoptosis. This response may create selective pressure that favors the survival or expansion of cells with pre-existing or acquired defects in TP53 or other DNA damage-response pathways, potentially raising concerns about abnormal clonal expansion and tumorigenic risk (181,182). Although recombinant adeno-associated virus (AAV) vectors predominantly persist as extrachromosomal episomes, vector-derived DNA can integrate into nuclease-induced DSBs. Such integration may involve full-length or fragmented vector sequences and can disrupt endogenous genes or alter their expression, depending on the integration site and vector sequence (183,184). These findings highlight the importance of assessing unintended genomic alterations and clonal stability when evaluating the long-term safety of genome editing therapies.At the transcriptomic level, while second-generation base editors avoid DSBs, they introduce transcriptome-wide off-target RNA deamination, necessitating the engineering of domain-inlaid Cas variants with narrowed editing windows to preserve transcriptomic stability (176).

Pre-existing and adaptive immunity to Cas protein. The clinical success of *in vivo* genome editing is influenced by host immune responses directed against both the delivery vehicle and the genome-editing machinery. Most commonly used CRISPR nucleases, including SpCas9 and SaCas9, are derived from bacterial species that interact with humans. Consequently, a notable proportion of the population possesses pre-existing adaptive immunity against these proteins (183-185). Both clinical and preclinical studies have demonstrated that systemic administration of CRISPR-based therapies may trigger humoral and cell immune responses (186). Pre-existing neutralizing antibodies can recognize and eliminate viral delivery vehicles, such as AAV vectors, before they reach target tissue, thereby decreasing therapeutic efficacy (177). In addition, intracellular expression of Cas proteins can result in antigen presentation through major histocompatibility complex class I (MHC-I) pathways, leading to activation of pre-existing cytotoxic $CD8^+$ T cells (187). These immune responses promote inflammation, decrease editing efficiency and contribute to the elimination of successfully edited cells. To overcome these immunological barriers, considerable efforts have focused on the development of transient delivery systems that minimize the duration of Cas protein exposure (188). Approaches such as engineered virus-like particles (eVLPs), selective organ-targeting (SORT)-LNPs and RNP delivery systems enable short-term nuclease activity while decreasing prolonged immune recognition (189). Furthermore, advances in protein engineering have facilitated the development of less immunogenic Cas variants through modification of dominant immune epitopes without compromising editing activity (190). Despite these advances, immunogenicity remains a challenge that must be addressed to ensure the safety and efficacy of CRISPR-based therapeutics (187).

Additional biological factors affecting editing outcomes. Several biological factors influence the performance of CRISPR-Cas systems. One of these is mosaicism, particularly in embryo editing and certain *in vivo* applications, where not all cells acquire the same genetic modification. This can decrease the consistency of editing outcomes and complicate interpretation of experimental results (191-193). Chromatin accessibility affects editing outcomes, because genomic regions differ in their accessibility to gRNA-Cas complexes. In addition, variation in DNA repair pathways between cell types and individuals may alter the balance between NHEJ and HDR. Population-specific genetic variation may also affect editing efficiency. Sequence variation within PAM regions or target sequences influences target recognition and editing fidelity (194). For this reason, broader evaluation of genome editing across genetically diverse populations remains important.

Remaining data gaps. Data gaps remain in the CRISPR literature. Long-term follow-up data from clinical studies remain limited, particularly for *in vivo* genome-editing approaches, creating uncertainty regarding the durability of therapeutic responses, delayed adverse events, and long-term safety (190,195). Although early clinical studies have demonstrated promising outcomes, extended monitoring is necessary to assess persistent editing effects, potential off-target alterations, and delayed toxicities. There are also gaps in the clinical evaluation of CRISPR-based therapies across disease areas. Much of the therapeutic development to date has focused on hematological disorders and cancer, whereas applications for neurodegenerative, autoimmune, and chronic inflammatory diseases remain less extensively evaluated in clinical settings (196,197). Additional work is needed to define tissue-specific editing thresholds, improve methods for measuring off-target effects and understand inter-individual variation in editing outcomes. These data gaps should be addressed before CRISPR-based therapies are applied more broadly and consistently in clinical practice.

Long-term safety considerations. Although CRISPR-based therapy demonstrated encouraging results in early clinical studies, the long-term safety profile remains incompletely understood. Because genome editing introduces permanent genetic modification, comprehensive evaluation of safety requires extended observation. While most current studies focus on short-term efficacy and immediate off-target effects, long-term risks remain under investigation (198).

One concern is the accumulation of delayed off-target effects. In delivery systems that support prolonged nuclease expression, such as certain viral vectors, persistent Cas activity may increase the opportunity for unintended DNA cleavage over time (190). These events may contribute to chromosomal rearrangements, large deletions, and other forms of genomic instability (110). Although adeno-associated virus (AAV) vectors predominantly persist as extrachromosomal episomes, vector-derived DNA can integrate at nuclease-induced DNA double-strand breaks. Depending on the integration site and the sequences inserted, such events may disrupt gene function or alter gene expression, potentially contributing to insertional mutagenesis (110,184). Another concern is the cellular response to CRISPR-induced double-strand breaks (DSBs). Activation of the p53-mediated DNA damage response can cause cell-cycle arrest or apoptosis, while the resulting selective pressure may favor the survival and expansion of cells with

impaired p53 function, raising concerns about abnormal clonal expansion and potential tumorigenic risk (183). Furthermore, the long-term persistence, stability, and biological behavior of edited cells remain incompletely characterized for many applications. Given these uncertainties, extended clinical surveillance is important for assessing therapeutic durability, delayed adverse events, and other unintended consequences. Risk-based, standardized long-term follow-up protocols are therefore essential for supporting the safe clinical translation of genome-editing therapies (198).

Logistical and biophysical bottlenecks [current good manufacturing practice (cGMP) and scale-up boundaries]. The final tier involves the physical constraints of large-scale biomanufacturing and clinical translation. Conventional adeno-associated virus (AAV) vectors have a limited packaging capacity of approximately 4.7 kb, including the inverted terminal repeats (ITRs), leaving less space for the therapeutic cassette itself. This constraint complicates the delivery of large genome-editing components, multiplexed editing systems, and long homology-directed repair (HDR) donor templates, because the editor, guide RNAs, regulatory elements, and donor sequences must fit within the available cargo space. Oversized AAV genomes may be packaged inefficiently or exhibit genome truncation, potentially compromising delivery performance. For transient non-viral NPs, packaging heavy macromolecular cargos such as Cas9 mRNA or pre-assembled RNP complexes weighing ~160 kDa presents biophysical scale-up hurdles (199). Achieving batch-to-batch reproducibility is complicated by the native solubility and aggregation behavior of the Cas9 protein inside formulation buffers. The presence of physical or thermal shear stressors in manufacturing equipment induces protein misfolding, dramatically lowering encapsulation efficiencies and nuclear localization capacity, while increasing host innate immune detection upon clinical administration (200).

Translational, regulatory and standardization challenges

Practical and regulatory barriers. Clinical translation of CRISPR-based therapies faces practical and regulatory barriers. Manufacturing of edited cells and gene editing reagents can be labor-intensive, expensive and difficult to scale for routine clinical use. These limitations are especially relevant for *ex vivo* therapies that require cell isolation, editing, expansion and quality control before reinfusion into patients (201).

Regulatory and ethical concerns remain, particularly in relation to germline editing, long-term follow-up and equitable access to treatment. International regulatory frameworks are not fully harmonized and differences in approval standards may slow the development and comparison of clinical studies (202). Another issue is the lack of standardized reporting for editing dose, treatment response and clinical outcome measures. Different metrics for delivery, editing efficiency and therapeutic success makes direct comparison difficult. Greater consistency in study design and reporting standards is necessary for future clinical development (203).

Structural dynamics of global regulatory heterogeneity and bioethics. The final boundary governing the clinical translation of CRISPR-Cas platforms encompasses macro-level institutional constraints: Global regulatory heterogeneity and ethical oversight. At the manufacturing and validation phase, chemistry, manufacturing and control requirements vary across regulatory bodies. Divergent cGMP standards between the US FDA and the European Medicines Agency regarding allowable *in silico* off-target prediction thresholds, deep-sequencing validation depth and AAV empty-to-full capsid ratios create major bottlenecks. These financial and administrative hurdles are particularly challenging for therapeutic development in rare diseases with small patient populations (204).

While somatic gene editing for severe, life-threatening conditions such as transfusion-dependent β-thalassemia and refractory solid tumors has achieved broad international consensus, a regulatory line is maintained against germline modifications and non-therapeutic genetic enhancement. The potential for introducing heritable, unverified off-target changes into the human gene pool poses evolutionary and sociological risks. Addressing these ethical concerns while accelerating clinical translation requires international consortia to establish unified, standardized frameworks for nuclease safety and manufacturing validation. Only through coordinated global harmonization can ethical governance keep pace with rapid advancements in biological engineering (205).

8. Emerging solutions: Engineering the next generation of CRISPR

Non-cleaving base and prime editing platforms. To bypass p53-mediated toxicity and chromosomal deletions, precision medicine is adopting base and prime editors. These systems combine a catalytically impaired Cas9 nickase or dCas9 with deaminase enzymes or reverse transcriptases. This configuration allows for the permanent conversion of single base pairs (mediating transition mutations such as C•G to T•A or A•T to G•C) or search-and-replace sequence modifications without generating DSBs. Advanced preclinical trials demonstrate that a single LNP-mediated delivery of these base editors corrects pathogenic point mutations in primate models (206,207), durably lowering disease markers without triggering long-term immune complications, establishing a non-disruptive paradigm for multi-decade genetic validation (208).

Advanced delivery platforms

Organ-tropic LNP engineering and SORT technology. To liberate LNPs from hepatic entrapment, rational design and high-throughput formulation screens are used to alter the internal organ affinity. The SORT framework demonstrates that the strategic incorporation of a supplementary, distinct lipid (anionic, cationic or ionizable) can systematically reprogram the organ-tropism of LNPs toward the lung, spleen or skeletal muscles without changing the primary formulation. Concurrently, surface-functionalization with targeting moieties such as transferrin receptor antibodies or low-density lipoprotein receptor-binding peptides exploits receptor-mediated transcytosis to shuttle LNPs across the BBB into the brain parenchyma (209).

eVLPs. Representing a structural convergence of viral entry efficiency and non-viral safety kinetics, eVLPs use viral structural scaffolds (retroviral group-specific antigen) or AAV capsids) devoid of viral oncogenic or replicative genetic material. Advanced structural engineering allows the direct physical

Table IX. Clustered regularly interspaced short palindromic repeats- CRISPR-associated proteinsystem delivery platforms

Platform	Advantages	Limitations	Optimal use
SORT-LNPs	Extrahepatic targeting, with high lung transduction efficiency (reported in ~65% of pulmonary endothelial cells)	Requires formulation optimization	Respiratory and splenic disorder
eVLPs	Near-zero off-target effects; transient RNP delivery	Complex manufacturing	Neuromuscular disorder
Evolved AAV capsids	~100-fold reduction in the required dose compared with conventional delivery methods, with the potential for BBB) penetration	Immunogenicity risk	Central nervous system and muscle disorder

SORT, selective organ targeting; LNP, lipid nanoparticle; eVLP, engineered virus-like particle; AAV, adeno-associated virus; BBB, blood-brain barrier.

packaging of pre-assembled Cas9/gRNA RNP complexes into the VLP lumen during biogenesis. Upon cell entry via native viral fusogenic mechanisms, the RNP cargo exerts immediate, highly efficient editing before undergoing rapid proteasomal degradation. This platform minimizes both insertional mutagenesis risk and off-target kinetics to near-zero, offering a highly translatable approach for neuromuscular disorder (210).

Directed evolution of high-tropic AAV capsids. High-throughput directed evolution and machine-learning-guided capsid engineering may improve the utility of viral vectors. Novel engineered variants selected in non-human primates (such as the BI-AAV lineages or AAV-PHP.eB) exhibit higher potency in crossing the BBB or penetrating deep myofibers via systemic administration compared with wild-type serotypes (AAV (211). This superior tropism allows a notable decrease (up to 100-fold) in the required clinical dosage, mitigating systemic immunogenicity and dose-dependent toxicity while maintaining high editing efficiency (162). Optimization of these delivery modalities is essential for translating CRISPR-based genome editing into safe and effective clinical therapies for challenging targets such as the CNS and skeletal muscle (Table IX) (212).

Anti-CRISPR (Acr) regulons as precision control tools

Small-molecule and optical off switches. Chemical and optical small-molecule controls (degraders, inhibitors or light-inducible split-Cas9 domains) acts as off-switches, allowing clinicians to terminate nuclease kinetics precisely following on-target cleavage (213).

Acr proteins. Bacteria and bacteriophages continuously evolve. While bacteria utilize CRISPR-Cas systems as adaptive immune defenses to restrict invading genetic elements, viruses have evolved counter-defense mechanisms to directly neutralize these systems. One such widespread mechanism involves Acr proteins, which function as potent biochemical inhibitors of CRISPR-Cas catalytic activity (214,215). Acr proteins were first identified in bacteriophages infecting *Pseudomonas* species; structural characterizations have shown that these proteins suppress CRISPR-mediated host immunity through distinct biochemical mechanisms, including direct steric blockade of DNA binding by Cas proteins or explicit interference with native endonuclease cleavage activity (Table X) (213,216).

High-resolution structural studies have characterized how Acr proteins inhibit CRISPR activity at the atomic level (217-220). Bondy-Denomy (214) identified unique Acr proteins that directly interfere with the assembly and formation of the active Cas9-DNA surveillance complex, thereby structurally preventing target DNA engagement and double-strand cleavage. Crystallographic evaluations have shown that specific Acr proteins bind directly to the functional (221), highly conserved domains of Cas9, including the HNH and RuvC nuclease domains, blocking their catalytic backbone hydrolysis (222). Acr proteins also dynamically influence gRNA loading and function. Certain anti-CRISPR variants, including Anti-CRISPR protein II-A4 (AcrIIA4) and Anti-CRISPR protein II-A6 (AcrIIA6), physically interfere with guide RNA loading and Cas9 activation. Structural studies have shown that AcrIIA6 occupies the RNA-binding channel of apo-Cas9, whereas AcrIIA4 stabilizes an inactive Cas9 conformation, preventing assembly of the functional Cas9-gRNA surveillance complex and subsequent target DNA recognition and cleavage (223).

Translational applications of Acr proteins. Because of their highly specific inhibitory kinetics, Acr proteins are actively being investigated as bioengineering tools for regulating human genome editing systems (224). The controlled, spatio-temporal deployment of Acr proteins represents a strategy to decrease unintended genome modifications during CRISPR-based somatic editing. By temporarily or programmatically inhibiting Cas enzyme activity after a defined kinetic window, these Acr proteins may limit cumulative off-target DNA cleavage, mitigate long-term genotoxicity and significantly improve the clinical safety and predictability of genome editing approaches in translational medicine.

9. CRISPR-mediated modulation of key signaling pathways in disease models

Role of CRISPR in the modulation of YAP activity in immune and tumor cells. CRISPR-based functional studies have

Table X. Mechanisms of Acr protein-mediated inhibition.

Mechanism	Description	Structural basis	Example Acr proteins
Steric blockade	Direct physical obstruction of DNA binding by Cas proteins	Acr proteins bind the PAM-interacting domain or DNA-binding groove, preventing target DNA engagement	AcrIIA2, AcrIIA4, AcrIIC1
Nuclease domain inhibition	Binds HNH or RuvC catalytic domains to block phosphodiester hydrolysis	Acr proteins occupy the active sites of endonuclease domains, preventing coordinated cleavage	AcrIIC3, AcrIIA5, AcrVA1
Guide RNA interference	Physical obstruction of guide RNA loading into apo-Cas architecture	Acr proteins bind the Cas protein surface that normally interacts with the crRNA-tracrRNA duplex	AcrIIA6, AcrVA5, AcrVIA1
Cas complex disruption	Prevention of multi-subunit effector complex assembly	Acr proteins bind key subunit interfaces, blocking functional complex formation	AcrIF1, AcrIF2, AcrIF3, AcrIE1

Acr, anti-CRISPR protein; Cas, CRISPR-associated protein; PAM, protospacer adjacent motif; HNH, histidine-asparagine-histidine nuclease domain; RuvC, RNase H-like nuclease domain; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; crRNA, CRISPR RNA; tracrRNA, trans-activating CRISPR RNA; apo-Cas, Cas protein in its RNA-free form.

identified the Hippo signaling pathway as an important regulator of tumor growth and cell survival. Components of this pathway, including Yes-associated protein 1 (YAP1), transcriptional co-activator with PDZ-binding motif (TAZ), and large tumor suppressor kinase 2 (LATS2), have been investigated using CRISPR-Cas9 knockout approaches in different cancer models (225,226). Loss of YAP1 activity is associated with reduced cell proliferation and increased apoptosis, supporting the role of Hippo signaling in tumor maintenance. YAP and TAZ also interact with other signaling pathways involved in tumor progression, including Wnt and TGF-β pathways. These interactions influence cell proliferation, epithelial-mesenchymal transition and survival. For this reason, CRISPR-mediated targeting of Hippo pathway components has been examined as a potential strategy for studying tumor biology and identifying therapeutic targets (227). YAP activity is linked to immune regulation within the tumor microenvironment. Experimental studies suggest that YAP can influence the expression of immune checkpoint molecules such as PD-L1 (228,229). Decreased YAP activity by genome editing has been associated with lower PD-L1 expression in certain experimental systems, which may improve tumor sensitivity to immune-based therapies (187). The molecular interactions within the Hippo-YAP signaling cascade and potential CRISPR intervention points are demonstrated in Fig. 7.

Editing NF-κB and p53 to regulate inflammation and apoptosis. The NF-κB and p53 pathways are key regulators of inflammation, cellular stress responses, cell-cycle control, and apoptosis. Alterations in these pathways are common in cancer and chronic inflammatory conditions. CRISPR-Cas9-based models have been used to investigate how disruption of genes within these pathways affects cytokine production, tumor-cell survival, and immune regulation. Experimental studies targeting RelA (the p65 subunit of NF-κB) and NF-κB1 have demonstrated that disruption of NF-κB signaling can reduce the expression of pro-inflammatory mediators, including interleukin-6 (IL-6) and TNF-α), although the effects depend on the cell type and experimental context (230,231). These findings suggest that CRISPR-mediated editing of NF-κB pathway components may be useful for studying the inflammatory environment that supports tumor progression.

CRISPR-based approaches have been used to examine the role of p53 in cancer models (232,233). Restoration or correction of TP53 function in TP53-deficient or mutant systems reactivate p53-dependent transcriptional programs (234), promote cell-cycle arrest or apoptosis, and suppress tumor-cell growth and other tumor-associated phenotypes (235). In addition, interactions between p53 loss and sustained NF-κB activity are linked to immune evasion and persistent tumor growth (224). The regulatory interactions between NF-κB signaling, p53 activity and CRISPR-mediated genome editing are illustrated in Fig. 8.

Modulation of immune escape by CRISPR-based approaches. Experimental studies suggest that alterations in NF-κB signaling modulate PD-L1 expression (236) and thereby influence the recruitment, intratumoral accumulation, and effector activity of $CD8^+$ T cells (237), contributing to tumor immune evasion (238). CRISPR-based studies have demonstrated that genetic perturbation of tumor-intrinsic pathways can alter PD-L1 expression and reshape $CD8^+$ T-cell infiltration and antitumor activity (239) CRISPR-based modification of genes involved in immune checkpoint regulation has therefore been used to study the association between tumor signaling pathways and anti-tumor immunity (240).

CRISPR-mediated disruption of NF-κB-associated genes has also been associated with changes in the expression of

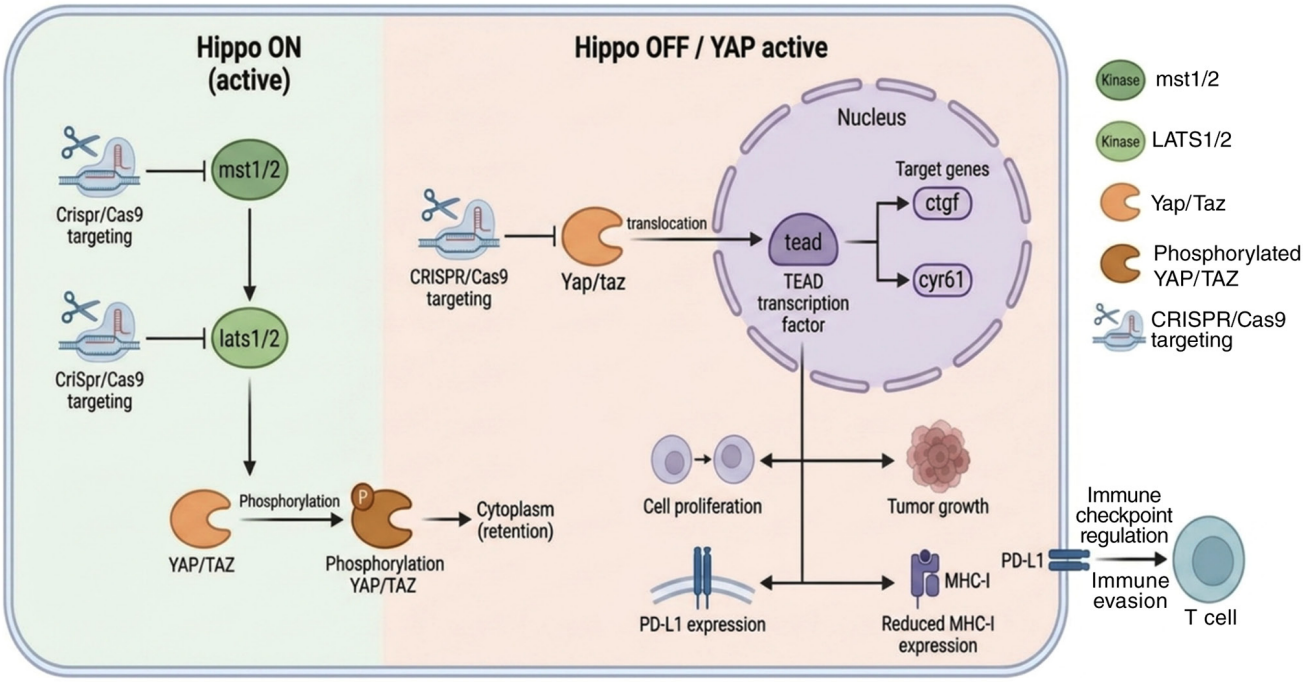


Figure 7. CRISPR-mediated modulation of the Hippo-YAP signaling pathway. The Hippo pathway regulates cell proliferation and immune signaling through phosphorylation-dependent control of YAP/TAZ transcriptional activity. Activation of MST1/2 and LATS1/2 kinases results in phosphorylation and cytoplasmic retention of YAP/TAZ, whereas pathway inactivation allows YAP/TAZ nuclear translocation and transcription of pro-growth genes such as CTGF and CYR61. CRISPR-based gene editing of Hippo pathway components modulates YAP activity, influencing tumor growth, PD-L1 expression, and immune checkpoint regulation. CRISPR, clustered regularly interspaced short palindromic repeats; YAP, yes-associated protein 1; TAZ, transcriptional coactivator with PDZ-binding motif; MST1/2, mammalian sterile 20-like kinase 1/2; LATS1/2, large tumor suppressor kinase 1/2; CTGF, connective tissue growth factor; CYR61, cysteine-rich angiogenic inducer 61; PD-L1, programmed death-ligand 1; Cas, CRISPR-associated protein; TEAD, TEA domain transcription factor; MHC-I, major histocompatibility complex class I.

MHC molecules and inflammatory cytokines. Decreased MHC expression impairs antigen presentation and weakens T cell recognition of tumor cells. Changes in cytokine profiles may promote an immunosuppressive tumor microenvironment (241). In addition, CRISPR has been applied to study the contribution of p53 to immune surveillance. Loss of p53 function is linked to impaired antigen presentation, reduced interferon signaling and decreased sensitivity to immune-mediated killing (242). Restoration of p53 activity in experimental models is associated with improved immune recognition (111,243). A schematic representation of CRISPR-based correction of immune evasion mechanisms is shown in Fig. 9.

10. Future perspectives

CRISPR-Cas systems may remain central tools in both biomedical research and therapeutic development, but barriers must be overcome before widespread clinical implementation becomes viable. Future progress depend on a coordinated effort across interconnected priority areas: Refinement of genome-editing platforms, development of tissue-specific delivery strategies, overcoming immunological barriers, manufacturing and regulatory standardization and expansion into underexplored disease areas (244).

First-generation CRISPR-Cas9 nucleases induce DSB with off-target frequencies of 0.1-5.0% depending on gRNA design and cell type. Newer platforms are addressing this limitation. High-fidelity Cas9 variants, including SpCas9-HF1, evoCas9 and HiFi-Cas9, have demonstrated 50- to 500-fold decreased off-target activity while maintaining therapeutic editing efficiency, with clinical trials expected to initiate in 2026-2027. Base editing, which enables permanent single-base conversion (C•G to T•A or A•T to G•C) without generating DSBs, has already entered Phase I trials for disorders including PCSK9-mediated hypercholesterolemia and transthyretin amyloidosis, with regulatory approval for select monogenic diseases projected for 2028-2030. Prime editing, a more versatile search-and-replace technology capable of all 12 base-to-base conversions and small insertions or deletions without donor DNA templates, remains in preclinical development but may enter clinical trials by 2027-2029. Epigenome editing, which modulates gene expression without altering the underlying DNA sequence, remains at an early stage and may require a longer development timeline beyond 2030. These refined platforms are well-suited for diseases caused by point mutations, including sickle cell disease, cystic fibrosis and numerous rare genetic disorders.

Parallel to editing platform refinement, the development of tissue-specific delivery strategies is required. Current delivery platforms exhibit strong hepatic tropism, with conventional lipid nanoparticles (LNPs) accumulating greater than 80% in the liver, while penetration across the blood-brain barrier (BBB) remains <1% without specialized surface engineering. To address this limitation, the selective organ targeting (SORT) framework adds a fifth lipid component to systematically reprogram nanoparticle organ tropism. This platform has achieved lung endothelial transduction of 65% and epithelial

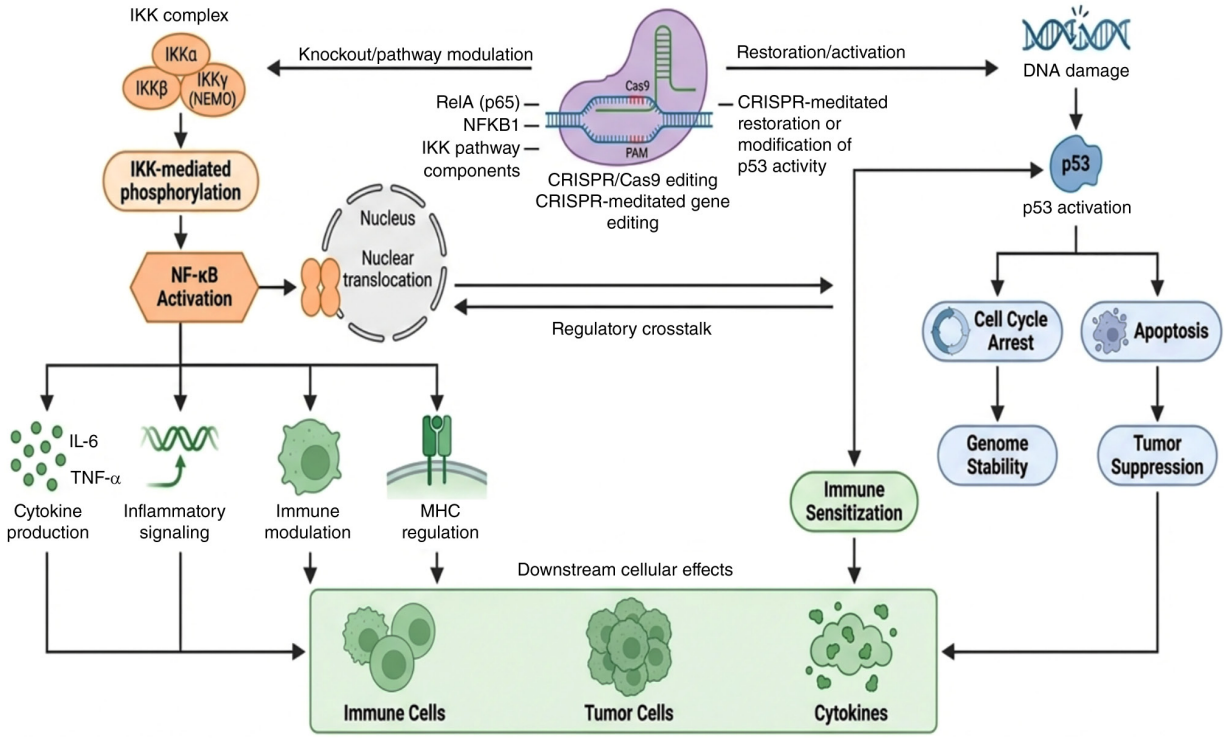


Figure 8. CRISPR-based regulation of NF-κB and p53 signaling pathways. Genome editing technologies modulate key signaling pathways involved in inflammation, immune regulation, and tumor suppression. CRISPR-mediated targeting of NF-κB pathway components influences cytokine production and inflammatory signaling, while editing of genes associated with the p53 pathway may restore tumor suppressor activity and promote cell cycle arrest or apoptosis. Crosstalk between NF-κB and p53 signaling contributes to immune regulation and tumor microenvironment dynamics. CRISPR, clustered regularly interspaced short palindromic repeats; IKK, inhibitor of nuclear factor kappa-B kinase; NEMO, NF-κB essential modulator; MHC, major histocompatibility complex; RelA, v-rel avian reticuloendotheliosis viral oncogene homolog A; Cas, CRISPR-associated protein; PAM, protospacer adjacent motif; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

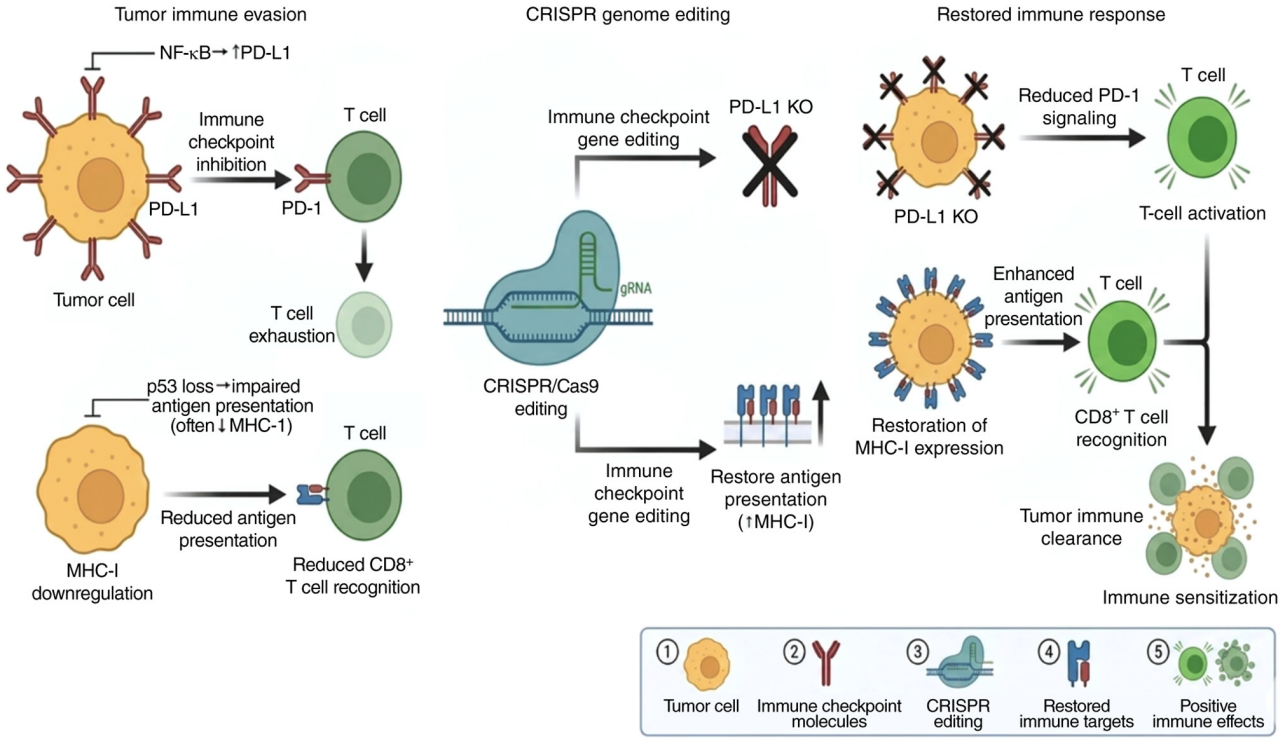


Figure 9. Schematic representation of CRISPR correction of immune evasion mechanisms. Tumor cells evade immune surveillance through immune checkpoint signaling and decreased antigen presentation, including PD-L1-mediated inhibition of T cell activity and downregulation of MHC-I expression. CRISPR-based genome editing targets immune checkpoint regulators such as PD-L1 and restores antigen presentation pathways. These modifications enhance $CD8^+$ T cell recognition of tumor cells, promote T cell activation, and support tumor immune clearance. CRISPR, clustered regularly interspaced short palindromic repeats; gRNA, guide RNA; PD-L1, programmed death-ligand 1; MHC-I, major histocompatibility complex class I; KO, knockout.

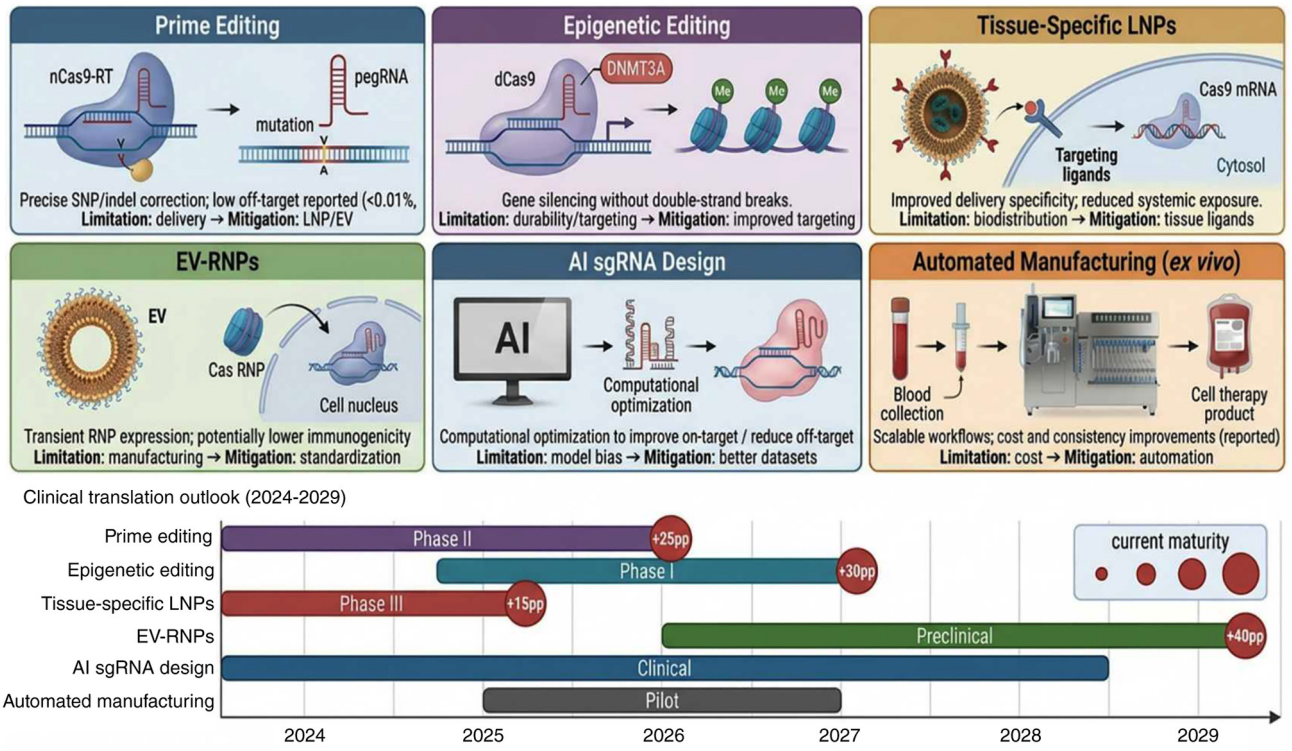


Figure 10. Emerging directions in CRISPR therapeutics, including prime and epigenetic editing, tissue-specific delivery systems, AI-assisted guide RNA design, EV-based delivery, and automated manufacturing. The horizontal timeline illustrates clinical translation tracks across the 2024-2029 observation window. AI, artificial intelligence; nCas9-RT, nickase Cas9-reverse transcriptase fusion protein; pegRNA, prime editing guide RNA; SNP, single-nucleotide polymorphism; indel, insertion or deletion; dCas9, catalytically inactive CRISPR-associated protein 9; DNMT3A, DNA methyltransferase 3 alpha; LNP, lipid nanoparticle; EV, extracellular vesicle; RNP, ribonucleoprotein; sgRNA, single-guide RNA; pp, percentage points.

cell transduction of 40% following a single low-dose injection, with clinical evaluation actively initiating and projected to expand through 2027.

Overcoming immunological barriers is key for successful clinical translation. Pre-existing adaptive immunity against SpCas9 affects 58-79% of healthy individuals and pre-existing neutralizing antibodies can eliminate viral delivery vehicles before they reach target tissue. Routine screening of patients for anti-Cas9 antibodies before treatment is immediately implementable and already adopted in clinical trials. Transient delivery platforms such as mRNA-formulated LNPs and eVLPs minimize the duration of Cas protein exposure, reducing the opportunity for adaptive immune recognition; these approaches are being evaluated in clinical trials for TTR amyloidosis and other liver-directed indications. Immunosuppressive preconditioning regimens are being optimized in preclinical models to temporarily dampen host responses against both delivery vectors and editing machinery. Finally, protein engineering efforts are focused on developing less immunogenic Cas variants through systematic modification of dominant immune epitopes while preserving catalytic activity. A combination of these approaches may required for successful systemic *in vivo* editing, particularly for indications requiring repeat administration.

Expansion into underexplored disease areas requires improved disease models, clearer therapeutic targets and better understanding of tissue-specific biology. Neurodegenerative disorders including Huntington's, Parkinson's and Alzheimer' disease remain largely untargeted by CRISPR due to the difficulty of efficient delivery across the BBB. However, evolved AAV capsids such as AAV-PHP.eB and BI-AAV lineages, as well as focused ultrasound-assisted delivery, offer promising avenues for central nervous system targeting. Autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis and type 1 diabetes require precise engineering of Treg populations without triggering off-target immune activation; CRISPR-based strategies to stabilize FOXP3 expression in Tregs are in preclinical development (245). Rare inherited diseases encompass more than 7,000 distinct disorders, the vast majority of which completely lack approved therapeutic options. While this immense landscape presents a critical frontier for clinical translation, restricted patient cohorts drastically undermine the economic viability of conventional pharmaceutical development. To circumvent these commercial bottlenecks, next-generation precision editing suites are being deployed; specifically, base and prime editing platforms are uniquely optimized to correct the single-nucleotide variations responsible for many rare pathologies. Furthermore, ongoing research into programmable, RNA-guided integrases holds promise for executing the large-scale genomic insertions required to restore full-length gene function in more complex structural defects (246).

The integration of artificial intelligence (AI) and machine learning (ML) into CRISPR research is accelerating progress across multiple fronts. Computational gRNA design platforms such as DeepCRISPR, CRISPRitz, and sgDesigner incorporate sequence characteristics and, in some approaches, epigenetic or chromatin-associated features to predict on-target activity and

potential off-target effects (125,162,247,248). Deep learning models trained on large-scale CRISPR screening datasets can improve gRNA activity prediction and prioritize candidate guides, thereby reducing the number of constructs requiring experimental testing, although experimental validation remains essential (249-251). Large-scale functional genomics resources, including the Cancer Dependency Map (DepMap), further provide extensive genetic-dependency datasets that can be integrated with computational models for target prioritization and cancer research (208). Future integration of AI with high-throughput screening data, patient genomic information, and protein-engineering datasets may facilitate therapeutic target discovery, improve context-specific gRNA selection, and help predict cellular or immunological responses to different Cas variants. Deep learning approaches are also increasingly being applied to genome-wide off-target prediction, enabling more comprehensive computational assessment of editing specificity and safety (252,253). Finally, the convergence of CRISPR-based genome editing with advances in bioprinting and tissue engineering holds promise for creating more sophisticated disease models and regenerative medicine applications. Patient-derived induced pluripotent stem cells edited using CRISPR-Cas9 can be incorporated into three-dimensional bioprinted tissue constructs, enabling the creation of personalized organoids that recapitulate disease phenotypes and drug responses. While this convergence remains in early stages, it suggests genome editing and tissue engineering are integrated to produce implantable, patient-specific tissue for the treatment of degenerative disease, traumatic injury and congenital abnormalities. Emerging four-dimensional bioprinting, which adds temporal control over construct shape and function, may enable the creation of dynamic tissue models that respond to environmental cues, further enhancing the predictive value of preclinical testing. In summary, the future of CRISPR-based therapy depends on integration of molecular innovation, delivery engineering, immunological management, manufacturing scalability, regulatory harmonization and ethical governance. Near-term approvals (2026-2028) is anticipated for liver-directed base editing and *ex vivo* editing for additional hematological disorders beyond β-thalassemia and sickle cell disease. Central nervous system and muscle disorder, while representing some of the most serious genetic diseases, will likely require longer development timelines (2030-2035) due to the challenges in delivery and long-term safety monitoring. Progress in these areas will ultimately determine how effectively genome editing can move from experimental use to wider therapeutic application and whether the promise of CRISPR-Cas systems can be realized equitably for patients across the full spectrum of human genetic disease. The rapid pace of advancement since the first CRISPR clinical trial in 2016 culminating in the first FDA approval of Casgevy in 2023 suggests barriers will be addressed, transforming what was once a bacterial defense system into a cornerstone of precision medicine (Fig. 10).

11. Conclusion

CRISPR-Cas9 has emerged as a transformative platform that has advanced the study of gene function, disease mechanisms and therapeutic development. The technology has enabled systematic identification of disease-associated genes through genome-wide screening, provided insights into molecular pathways and facilitated the generation of advanced disease models ranging from patient-derived organoids to multiplex-edited large animals. In diagnostics, CRISPR-based platforms have achieved attomolar sensitivity for pathogen detection, with clinical validation for SARS-CoV-2, Zika and Dengue. In therapeutics, Casgevy received FDA approval in December 2023 for transfusion-dependent β-thalassemia and sickle cell disease. CRISPR-modified CAR-T cells have shown promising responses in hematological malignancies, establishing CRISPR-Cas as a clinically validated modality. Despite these achievements, barriers remain before widespread clinical implementation becomes viable. Off-target effects, detectable at 0.1-5.0% frequency, risk oncogene activation and chromosomal instability. Delivery to non-hepatic tissue remains inefficient; standard LNPs exhibit >80% hepatic tropism, while BBB penetration is <1%. Pre-existing immunity against Cas9 affects 58-79% of healthy individuals, potentially neutralizing efficacy and precluding repeat dosing. HDR for large-fragment gene correction remains inefficient (<5%) in non-dividing cells. Long-term safety data are incomplete; delayed adverse events may not manifest for decades. Promising solutions are emerging: High-fidelity Cas variants achieve 50- to 500-fold reductions in off-target activity. Base and prime editing enable precise single-base conversion without DSB, bypassing p53-mediated toxicity. Tissue-specific delivery platforms SORT-LNPs, eVLPs and evolved AAV capsids offer targeting precision for challenging tissue such as the CNS and skeletal muscle. Acr off-switches provide temporal control of nuclease activity. However, these technologies require clinical validation. The future of CRISPR-based medicine depends not only on maximizing efficacy, but on achieving an acceptable risk-benefit balance for each indication. For severe, life-threatening diseases with no alternative treatments, the current risk profile may be justified. For less severe conditions, improved safety profiles are required. Near-term approvals are anticipated for liver-directed base editing and additional *ex vivo* indications, while CNS and muscle disorder require longer timelines due to delivery and safety challenges. CRISPR-Cas technology has made contributions to biological research and are increasingly shaping gene-based therapy. Continued progress in delivery, precision, manufacturing and long-term safety monitoring may determine how effectively genome editing moves from experimental use to wider therapeutic application. The CRISPR-Cas system is an integrated clinical translation ecosystem connecting basic disease models with rapid diagnosis and therapeutic intervention across different stages of clinical maturity.

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Authors' contributions

Data authentication is not applicable. AA and MOI conceived and designed the review, performed the literature search, constructed figures and wrote the initial manuscript. MOI contributed to literature analysis, manuscript organization, and critical revision of the manuscript. YG assisted with literature interpretation and manuscript refinement. JC supervised the project, provided critical scientific guidance, and revised the manuscript. BZ contributed to scientific discussion, manuscript evaluation, and final approval of the submitted version. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ganger S, Harale G and Majumdar P: Clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) systems: Discovery, structure, classification, and general mechanism. In: CRISPR/Cas-Mediated Genome Editing in Plants. Apple Academic Press, Palm Bay, FL, p33, 2023.
2. Davis DJ and Yeddula SGR: CRISPR advancements for human health. Mo Med 121: 170-176, 2024.
3. Liao H, Wu J, VanDusen NJ, Li Y and Zheng Y: CRISPR-Cas9-mediated homology-directed repair for precise gene editing. Mol Ther Nucleic Acids 35: 102344, 2024.
4. Far BF, Akbari M, Habibi MA, Katavand M and Nasseri S: CRISPR technology in disease management: An updated review of clinical translation and therapeutic potential. Cell Prolif 58: e70099, 2025.
5. Mustafa M and Makhawi A: SHERLOCK and DETECTR: CRISPR-Cas systems as potential rapid diagnostic tools for emerging infectious diseases. J Clin Microbiol 59: e00745-20, 2021.
6. Louwen R, Staals RH, Endtz HP, van Baarlen P and van der Oost J: The role of CRISPR-Cas systems in virulence of pathogenic bacteria. Microbiol Mol Biol Rev 78: 74-88, 2014.
7. Jansen R, Embden JD, Gaastra W and Schouls LM: Identification of genes that are associated with DNA repeats in prokaryotes. Mol Microbiol 43: 1565-1575, 2002.
8. Mojica FJ, Díez-Villaseñor C, García-Martínez J and Soria E: Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol 60: 174-182, 2005.
9. Clarke R, Terry AR, Pennington H, Hasty C, MacDougall MS, Regan M and Merrill BJ: Sequential activation of guide RNAs to enable successive CRISPR-Cas9 activities. Mol Cell 81: 226-238. e5, 2021.
10. Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, Barrangou R, Brouns SJ, Charpentier E, Haft DH, *et al*: An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol 13: 722-736, 2015.
11. Weinberger AD, Sun CL, Pluciński MM, Denef VJ, Thomas BC, Horvath P, Barrangou R, Gilmore MS, Getz WM and Banfield JF: Persisting viral sequences shape microbial CRISPR-based immunity. PLoS Comput Biol 8: e1002475, 2012.
12. Ganguly C, Rostami S, Long K, Aribam SD and Rajan R: Unity among the diverse RNA-guided CRISPR-Cas interference mechanisms. J Biol Chem 300: 107295, 2024.
13. Yang P, Zhang S, Hu D, Li X, Guo Y, Guo H, Zhang L and Ding X: Research progress on the mechanism and application of the type I CRISPR-Cas system. Int J Mol Sci 25: 12544, 2024.
14. Levy JM, Yeh WH, Pendse N, Davis JR, Hennessey E, Butcher R, Koblan LW, Comander J, Liu Q and Liu DR: Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses. Nat Biomed Eng 4: 97-110, 2020.
15. Alshorman J, Mehran MJ, Miyanda Tembo K, Mostafavi N, Bolideei M and Wang Y: Applications of genome editing technologies in the treatment of human diseases. Gene Ther: Jun 15, 2026 (Epub ahead of print).
16. Lai Y, Yue Y and Duan D: Evidence for the failure of adeno-associated virus serotype 5 to package a viral genome > or = 8.2 kb. Mol Ther 18: 75-79, 2010.
17. Chen T, Hu G, Fu J and Tu J: Structural basis of PAM-Induced conformational changes in SpCas9: A molecular dynamics study. J Chem Inf Model 65: 10624-10633, 2025.
18. Broothaerts W, Jacchia S, Angers A, Petrillo M, Querci M, Savini C and Emons H: New genomic techniques: State-of-the-art review. Publications Office of the European Union, Luxembourg, 2021.
19. Xu Y and Li Z: CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J 18: 2401-2415, 2020.
20. Friedland AE, Baral R, Singhal P, Loveluck K, Shen S, Sanchez M, Marco E, Gotta GM, Maeder ML, Kennedy EM, *et al*: Characterization of Staphylococcus aureus Cas9: A smaller Cas9 for all-in-one adeno-associated virus delivery and paired nickase applications. Genome Biol 16: 257, 2015.
21. Greco F, Cosentino M and Marino F: The Italian breakthrough in CRISPR trials for rare diseases: A focus on beta-thalassemia and sickle cell disease treatment. Front Med (Lausanne) 11: 1356578, 2024.
22. Chakraborty C, Bhattacharya M, Das A, Agoramoorthy G and Lee SS: CRISPR-Cas9-mediated therapeutics: Current clinical trials and therapy approval landscape to treat human diseases. Mol Ther Nucleic Acids 37: 102859, 2026.
23. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, *et al*: Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163: 759-771, 2015.
24. Cheng H, Jeong E and Cho SW: Applications of multiplexed CRISPR-Cas for genome engineering. Exp Mol Med 57: 1373-1380, 2025.
25. Ji Z, Zhao Y, Huang J, Shan Y, Li J, Huang Q, Wu M and Liu F: Portable amplification-free digital droplet CRISPR/Cas12a platform for one-pot multiplexed viruses detection with attomolar sensitivity. Biosens Bioelectron 305: 118668, 2026.
26. Kordyś M, Sen R and Warkocki Z: Applications of the versatile CRISPR-Cas13 RNA targeting system. Wiley Interdiscip Rev RNA 13: e1694, 2022.
27. Tan X, Li J, Cui B, Wu J, Toischer K, Hasenfuß G and Xu X: CRISPR/Cas13-based anti-RNA viral approaches. Genes (Basel) 16: 875, 2025.
28. Pavlović M, Knežić Avramov T, Avramov M, Popović Ž, Gadjanski I and Djisalov M: Recent advances in isothermal strategies for biomedical molecular diagnostics. Trendovi u molekularnoj Biologiji 5: 156-187, 2025.
29. Tarique MTB: CRISPR-Cas systems in antiviral and antimicrobial defence: Recent advances, challenges, and biodefense implications. BRAC University, 2025. Available from: http://hdl.handle.net/10361/27538.
30. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA and Charpentier E: A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821, 2012.
31. Park SJ, Lee GE, Cho SM and Choi EH: Recent applications, future perspectives, and limitations of the CRISPR-Cas system. Mol Ther Nucleic Acids 36: 102634, 2025.

32. Punde A, Dey S, Pandire R, Bhattacharjee A and Patra C: Expanding the CRISPR/Cas toolkit: Applications in proteomics and theranostics. Front Bioeng Biotechnol 13: 1713700, 2025.
33. Ma D, Xu Z, Zhang Z, Chen X, Zeng X, Zhang Y, Deng T, Ren M, Sun Z, Jiang R and Xie Z: Engineer chimeric Cas9 to expand PAM recognition based on evolutionary information. Nat Commun 10: 560, 2019.
34. Casalino L, Nierzwicki Ł, Jinek M and Palermo G: Catalytic mechanism of non-target DNA cleavage in CRISPR-Cas9 revealed by ab initio molecular dynamics. ACS Catal 10: 13596-13605, 2020.
35. Allemailem KS, Almatroudi A, Rahmani AH, Alrumaihi F, Alradhi AE, Alsubaiyel AM, Algahtani M, Almousa RM, Mahzari A, Sindi AAA, *et al*: Recent updates of the CRISPR/Cas9 genome editing system: Novel approaches to regulate its spatio-temporal control by genetic and physicochemical strategies. Int J Nanomedicine 19: 5335-5363, 2024.
36. Obafemi OT, Ayeleso AO, Adewale OB, Unuofin J, Ekundayo BE, Ntwasa M and Lebelo SL: Animal models in biomedical research: Relevance of Drosophila melanogaster. Heliyon 11: e41605, 2024.
37. Bezard E: Non-human primate models of neurodegenerative diseases: Advancing Translational Research. Revue de primatologie, 2025. Available from: https://doi.org/10.4000/14md7.
38. Albitar A, Rohani B, Will B, Yan A and Gallicano GI: The application of CRISPR/Cas technology to efficiently model complex cancer genomes in stem cells. J Cell Biochem 119: 134-140, 2018.
39. Tu Z, Yang W, Yan S, Guo X and Li XJ: CRISPR/Cas9: A powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. Mol Neurodegener 10: 35, 2015.
40. Jacinto FV, Link W and Ferreira BI: CRISPR/Cas9-mediated genome editing: From basic research to translational medicine. J Cell Mol Med 24: 3766-3778, 2020.
41. Gridley T and Murray SA: Mouse mutagenesis and phenotyping to generate models of development and disease. Curr Top Dev Biol 148: 1-12, 2022.
42. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F and Jaenisch R: One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153: 910-918, 2013.
43. Eaton SL and Wishart TM: Bridging the gap: Large animal models in neurodegenerative research. Mamm Genome 28: 324-337, 2017.
44. Park JE and Silva AC: Generation of genetically engineered non-human primate models of brain function and neurological disorders. Am J Primatol 81: e22931, 2019.
45. Akyuz E, Aslan FS, Gokce E, Ilmaz O, Topcu F and Kakac S: Extracellular vesicle and CRISPR gene therapy: Current applications in Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. Eur J Neurosci 60: 6057-6090, 2024.
46. Pacesa M, Pelea O and Jinek M: Past, present, and future of CRISPR genome editing technologies. Cell 187: 1076-1100, 2024.
47. Bassi G, Grimaudo MA, Panseri S and Montesi M: Advanced multi-dimensional cellular models as emerging reality to reproduce in vitro the human body complexity. Int J Mol Sci 22: 1195, 2021.
48. Madan A, Saini R, Dhiman N, Juan SH and Satapathy MK: Organoids as next-generation models for tumor heterogeneity, personalized therapy, and cancer research: Advancements, applications, and future directions. Organoids 4: 23, 2025.
49. Tong L, Cui W, Zhang B, Fonseca P, Zhao Q, Zhang P, Xu B, Zhang Q, Li Z, Seashore-Ludlow B, *et al*: Patient-derived organoids in precision cancer medicine. Med 5: 1351-1377, 2024.
50. Gungor-Ozkerim PS, Inci I, Zhang YS, Khademhosseini A and Dokmeci MR: Bioinks for 3D bioprinting: An overview. Biomater Sci 6: 915-946, 2018.
51. Bonetti L and Scalet G: 4D fabrication of shape-changing systems for tissue engineering: State of the art and perspectives. Prog Addit Manuf 10: 1913-1943, 2025.
52. Heinzelmann E, Piraino F, Costa M, Roch A, Norkin M, Garnier V, Homicsko K and Brandenberg N: iPSC-derived and Patient-Derived Organoids: Applications and challenges in scalability and reproducibility as pre-clinical models. Curr Res Toxicol 7: 100197, 2024.
53. Tavakoli K, Pour-Aboughadareh A, Kianersi F, Poczai P, Etminan A and Shooshtari L: Applications of CRISPR-Cas9 as an advanced genome editing system in life sciences. BioTech (Basel) 10: 14, 2021.
54. Bahrulolum H, Tarrahimofrad H, Rouzbahani FN, Nooraei S, Sameh MM, Hajizade A and Ahmadian G: Potential of CRISPR/Cas system as emerging tools in the detection of viral hepatitis infection. Virol J 20: 91, 2023.
55. Lou J, Wang B, Li J, Ni P, Jin Y, Chen S, Xi Y, Zhang R and Duan G: The CRISPR-Cas system as a tool for diagnosing and treating infectious diseases. Mol Biol Rep 49: 11301-11311, 2022.
56. Shariq M, Khan MF, Raj R, Ahsan N, Singh R and Kumar P: CRISPR-based diagnostic approaches: Implications for rapid management of future pandemics. Mol Med Rep 27: 118, 2023.
57. Weng Z, You Z, Yang J, Mohammad N, Lin M, Wei Q, Gao X and Zhang Y: CRISPR-Cas biochemistry and CRISPR-based molecular diagnostics. Angew Chem Int Ed Engl 62: e202214987, 2023.
58. Tian Y, Li C, Zhao E, Chen Y, Shen X, Xu S, Yu Y and Sun L: Recent Advances in the Detection of Plant Diseases Based on the CRISPR-Cas System. Anal Chem 98: 13951-13966, 2026.
59. Ghouneimy A, Mahas A, Marsic T, Aman R and Mahfouz M: CRISPR-based diagnostics: Challenges and potential solutions toward point-of-care applications. ACS Synth Biol 12: 1-16, 2023.
60. Pan Z, Xu L, Fan Z, Cao Y and Ren F: CRISPR-based diagnostics for infectious diseases: Mechanisms, advancements and clinical transformation prospects. Front Cell Infect Microbiol 16: 1769226, 2026.
61. Tiwari P and Rathinasabapathi P: Programmable CRISPR-Cas diagnostic platforms for rapid detection of uropathogens and antimicrobial resistance. Arch Microbiol 208: 344, 2026.
62. Chakraborty J, Chaudhary AA, Khan SU, Rudayni HA, Rahaman SM and Sarkar H: CRISPR/Cas-based biosensor as a new age detection method for pathogenic bacteria. ACS Omega 7: 39562-39573, 2022.
63. Kaur R, Gupta S, Chauhan A, Mishra V, Sharma MK and Singh J: Harnessing the power of clustered regularly interspaced short palindromic repeats (CRISPR) based microfluidics for next-generation molecular diagnostics. Mol Biol Rep 51: 896, 2024.
64. Xie S, Yue Y and Yang F: Recent advances in CRISPR/Cas system-based biosensors for the detection of foodborne pathogenic microorganisms. Micromachines (Basel) 15: 1329, 2024.
65. Wang M, Zhang R and Li J: CRISPR/cas systems redefine nucleic acid detection: Principles and methods. Biosens Bioelectron 165: 112430, 2020.
66. Kaminski MM, Abudayyeh OO, Gootenberg JS, Zhang F and Collins JJ: CRISPR-based diagnostics. Nat Biomed Eng 5: 643-656, 2021.
67. He Y, Tu X, Xue Y, Chen Y, Ye B, Li X, Li D, Zhong Z and Zhong Q: CRISPR screening redefines therapeutic target identification and drug discovery with precision and scalability. J Pharm Anal 16: 101357, 2026.
68. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen T, Heckl D, Ebert BL, Root DE, Doench JG and Zhang F: Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343: 84-87, 2014.
69. Le Rhun A, Escalera-Maurer A, Bratovič M and Charpentier E: CRISPR-Cas in streptococcus pyogenes. RNA Biol 16: 380-389, 2019.
70. Nambiar TS, Baudrier L, Billon P and Ciccia A: CRISPR-based genome editing through the lens of DNA repair. Mol Cell 82: 348-388, 2022.
71. Zhang F, Wen Y and Guo X: CRISPR/Cas9 for genome editing: Progress, implications and challenges. Hum Mol Genet 23(R1): R40-R46, 2014.
72. Lone BA, Karna SKL, Ahmad F, Shahi N and Pokharel YR: CRISPR/Cas9 system: A bacterial tailor for genomic engineering. Genet Res Int 2018: 3797214, 2018.
73. Evers B, Jastrzebski K, Heijmans JP, Grernrum W, Beijersbergen RL and Bernards R: CRISPR knockout screening outperforms shRNA and CRISPRi in identifying essential genes. Nat Biotechnol 34: 631-633, 2016.
74. Liang L and Wang W: The impact of p53 mutation on tumor immune evasion: Mechanistic insights and clinical implications. Front Immunol 17: 1753215, 2026.
75. McLaughlin JE, Kue Foka IC, Lawton MA and Di R: CRISPR activation: Identifying and using novel genes for plant disease resistance breeding. Front Genome Ed 7: 1596600, 2025.
76. Zhang Z, Wang H, Yan Q, Cui J, Chen Y, Ruan S, Yang J, Wu Z, Han M, Huang S, *et al*: Genome-wide CRISPR/Cas9 screening for drug resistance in tumors. Front Pharmacol 14: 1284610, 2023.
77. Garg P, Pareek S, Kulkarni P, Horne D, Salgia R and Singhal SS: Next-generation immunotherapy: Advancing clinical applications in cancer treatment. J Clin Med 13: 6537, 2024.

78. Vaghari-Tabari M, Hassanpour P, Sadeghsoltani F, Malakoti F, Alemi F, Qujeq D, Asemi Z and Yousefi B: CRISPR/Cas9 gene editing: A new approach for overcoming drug resistance in cancer. Cell Mol Biol Lett 27: 49, 2022.
79. Zhang XH, Tee LY, Wang XG, Huang QS and Yang SH: Off-target effects in CRISPR/Cas9-mediated genome engineering. Mol Ther Nucleic Acids 4: e264, 2015.
80. Wu Y, Wang Y, Wang Y, Qiu H, Yuan X, Xiong H and Zou Y: Exploring synthetic lethality in cancer therapy: CRISPR-Cas9 technology offers new hope. Biochim Biophys Acta Rev Cancer 1880: 189370, 2025.
81. Xu Y, Chen C, Guo Y, Hu S and Sun Z: Effect of CRISPR/Cas9-edited PD-1/PD-L1 on tumor immunity and immunotherapy. Front Immunol 13: 848327, 2022.
82. Li W, Xu H, Xiao T, Cong L, Love MI, Zhang F, Irizarry RA, Liu JS, Brown M and Liu XS: MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biol 15: 554, 2014.
83. Tian S, Qin Y, Wu Y and Dong M: Design, performance, processing, and validation of a pooled CRISPR perturbation screen for bacterial toxins. Nat Protoc 20: 1158-1195, 2025.
84. Uddin F, Rudin CM and Sen T: CRISPR gene therapy: Applications, limitations, and implications for the future. Front Oncol 10: 1387, 2020.
85. Alagoz M and Kherad N: Advance genome editing technologies in the treatment of human diseases: CRISPR therapy (Review). Int J Mol Med 46: 521-534, 2020.
86. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez ML and Barrera-Saldaña HA: Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases (Review). Int J Mol Med 43: 1559-1574, 2019.
87. Zang SS, Zhang R, Zhang JR, Zhang X and Li J: Progress, applications and prospects of CRISPR-based genome editing technology in gene therapy for cancer and sickle cell disease. Hum Gene Ther 36: 858-869, 2025.
88. Sen T and Thummer RP: CRISPR and iPSCs: Recent developments and future perspectives in neurodegenerative disease modelling, research, and therapeutics. Neurotox Res 40: 1597-1623, 2022.
89. Cavazzana M, Antoniani C and Miccio A: Gene therapy for β-hemoglobinopathies. Mol Ther 25: 1142-1154, 2017.
90. Zeng S, Lei S, Qu C, Wang Y, Teng S and Huang P: CRISPR/Cas-based gene editing in therapeutic strategies for beta-thalassemia. Hum Genet 142: 1677-1703, 2023.
91. Li L and Mandal PK: Recent advancements in gene therapy for sickle cell disease and β-thalassemia. Front. Hematol 3: 1468952, 2024.
92. Liu R, Cong S, Gao Y, Xu J and Shi X: Application of CRISPR-Cas9-Based gene editing technology in inherited liver diseases. Int J Mol Sci 27: 6469, 2026.
93. Jacob EM, Borah A and Sakthi Kumar D: CRISPR/Cas9 Nanodelivery approaches for targeted gene therapy. In: Pharmaceutical Nanobiotechnology for Targeted Therapy. Springer International Publishing, Cham, pp27-64, 2022.
94. Ng CX, Mustafa S, Yap XY and Lee SH: CRISPR/Cas9 in cancer therapy: Clinical translation, mechanistic strategies, and therapeutic directions. Front Oncol 16: 1888734, 2026.
95. Chehelgerdi M, Chehelgerdi M, Khorramian-Ghahfarokhi M, Shafieizadeh M, Mahmoudi E, Eskandari F, Rashidi M, Arshi A and Mokhtari-Farsani A: Comprehensive review of CRISPR-based gene editing: Mechanisms, challenges, and applications in cancer therapy. Mol Cancer 23: 9, 2024.
96. Tao R, Han X, Bai X, Yu J, Ma Y, Chen W, Zhang D and Li Z: Revolutionizing cancer treatment: enhancing CAR-T cell therapy with CRISPR/Cas9 gene editing technology. Front Immunol 15: 1354825, 2024.
97. Lyons-Weiler J: Who are We, and Who (or What) Do We Want to Become? An Evolutionary Perspective on Biotransformative Technologies. Biological Theory 17: 138-152, 2022.
98. Alves E, Taifour S, Dolcetti R, Chee J, Nowak AK, Gaudieri S and Blancafort P: Reprogramming the anti-tumor immune response via CRISPR genetic and epigenetic editing. Mol Ther Methods Clin Dev 21: 592-606, 2021.
99. Liu Q: World-first phase I clinical trial for CRISPR-Cas9 PD-1-edited T-cells in advanced nonsmall cell lung cancer. Glob Med Genet 7: 73-74, 2020.
100. He S: The first human trial of CRISPR-based cell therapy clears safety concerns as new treatment for late-stage lung cancer. Signal Transduct Target Ther 5: 168, 2020.
101. Li X, Li Y, Dong L, Chang Y, Zhang X, Wang C, Chen M, Bo X, Chen H, Han W and Nie J: Decitabine priming increases anti-PD-1 antitumor efficacy by promoting CD8+ progenitor exhausted T cell expansion in tumor models. J Clin Invest 133: e165673, 2023.
102. Norberg SM and Hinrichs CS: Engineered T cell therapy for viral and non-viral epithelial cancers. Cancer Cell 41: 58-69, 2023.
103. Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, Huang M, Yi X, Liang M, Wang Y, *et al*: Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nat Med.26: 732-740, 2020.
104. Li C, Mei H and Hu Y: Applications and explorations of CRISPR/Cas9 in CAR T-cell therapy. Brief Funct Genomics 19: 175-182, 2020.
105. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, Odak A, Gönen M and Sadelain M: Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 543: 113-117, 2017.
106. Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, Mangan PA, Kulikovskaya I, Gupta M, Chen F, *et al*: CRISPR-engineered T cells in patients with refractory cancer. Science 367: eaba7365, 2020.
107. Agarwal S, Aznar MA, Rech AJ, Good CR, Kuramitsu S, Da T, Gohil M, Chen L, Hong SA, Ravikumar P, *et al*: Deletion of the inhibitory co-receptor CTLA-4 enhances and invigorates chimeric antigen receptor T cells. Immunity 56: 2388-2407.e9, 2023.
108. Zhang J, Hu Y, Yang J, Li W, Zhang M, Wang Q, Zhang L, Wei G, Tian Y, Zhao K, *et al*: Non-viral, specifically targeted CAR-T cells achieve high safety and efficacy in B-NHL. Nature 609: 369-374, 2022.
109. Zhang C, Liu J, Zhong JF and Zhang X: Engineering CAR-T cells. Biomark Res 5: 22, 2017.
110. Leibowitz ML, Papathanasiou S, Doerfler PA, Blaine LJ, Sun L, Yao Y, Zhang CZ, Weiss MJ and Pellman D: Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat Genet 53: 895-905, 2021.
111. Chen X, Zhong S, Zhan Y and Zhang X: CRISPR-Cas9 applications in T cells and adoptive T cell therapies. Cell Mol Biol Lett 29: 52, 2024.
112. Lin H, Li G, Peng X, Deng A, Ye L, Shi L, Wang T and He J: The use of CRISPR/Cas9 as a tool to study human infectious viruses. Front Cell Infect Microbiol 11: 590989, 2021.
113. Moso MA, Roche M, Cevaal PM and Lewin SR: CRISPR/Cas9 for achieving postintervention HIV control. Curr Opin HIV AIDS 20: 432-440, 2025.
114. Liao HK, Gu Y, Diaz A, Marlett J, Takahashi Y, Li M, Suzuki K, Xu R, Hishida T, Chang CJ, *et al*: Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nat Commun 6: 6413, 2015.
115. Rath JA and Arber C: Engineering strategies to enhance TCR-based adoptive T cell therapy. Cells 9: 1485, 2020.
116. Osborn MJ, Webber BR, Knipping F, Lonetree CL, Tennis N, DeFeo AP, McElroy AN, Starker CG, Lee C, Merkel S, *et al*: Evaluation of TCR gene editing achieved by TALENs, CRISPR/Cas9, and megaTAL nucleases. Mol Ther 24: 570-581, 2016.
117. Rahal Z, El Darzi R, Moghaddam SJ, Cascone T and Kadara H: Tumour and microenvironment crosstalk in NSCLC progression and response to therapy. Nat Rev Clin Oncol 22: 463-482, 2025.
118. Armillotta MG, Lizzi L and Massimi M: Nanoparticle-based systems for liver therapy: Overcoming fibrosis and enhancing drug efficacy. World J Hepatol 17: 108810, 2025.
119. Chen Y, Niu S, Li YR and Yang L: Innovative gene engineering strategies to address tumor antigen escape in cell therapy. J Transl Med 23: 1227, 2025.
120. Safarzadeh Kozani P and Safarzadeh Kozani P: Preventing secondary primary malignancies (SPMs) in CAR-T cell therapy through site-specific transgene integration into genomic safe harbors (GSHs). J Transl Med 23: 1155, 2025.
121. Dash PK, Chen C, Kaminski R, Su H, Mancuso P, Sillman B, Zhang C, Liao S, Sravanam S, Liu H, *et al*: CRISPR editing of CCR5 and HIV-1 facilitates viral elimination in antiretroviral drug-suppressed virus-infected humanized mice. Proc Natl Acad Sci USA 120: e2217887120, 2023.
122. Biederstädt A, Manzar GS and Daher M: Multiplexed engineering and precision gene editing in cellular immunotherapy. Front Immunol 13: 1063303, 2022.

123. Li MF, Zubair A, Wdidi S and He S: Disrupting viral persistence: CRISPR/Cas9-Based strategies for hepatitis B and C treatment, and challenges. J Cell Mol Med 30: e70986, 2026.
124. Mohamed H, Gurrola T, Berman R, Collins M, Sariyer IK, Nonnemacher MR and Wigdahl B: Targeting CCR5 as a component of an HIV-1 therapeutic strategy. Front Immunol 12: 816515, 2022.
125. Cortez JT, Montauti E, Shifrut E, Gatchalian J, Zhang Y, Shaked O, Xu Y, Roth TL, Simeonov DR, Zhang Y, *et al*: CRISPR screen in regulatory T cells reveals modulators of Foxp3. Nature 582: 416-420, 2020.
126. Ahmad S, Aftab MN and Aslam MS: From discovery to precision tolerance: Re-Imagining patient-matched regulatory T cells in shaping of a roadmap for personalized immune Re-balancing. Hum Immunol 87: 111747, 2026.
127. Strich JR and Chertow DS: CRISPR-Cas biology and its application to infectious diseases. J Clin Microbiol 57: e01307-18, 2019.
128. Li H, Yang Y, Hong W, Huang M, Wu M and Zhao X: Applications of genome editing technology in the targeted therapy of human diseases: Mechanisms, advances and prospects. Signal Transduct Target Ther 5: 1, 2020.
129. Radhakrishnan SK, Layden TJ and Gartel AL: RNA interference as a new strategy against viral hepatitis. Virology 323: 173-181, 2004.
130. Raigani M, Eftekhari Z, Adeli A and Kazemi-Lomedasht F: Advancing gene editing therapeutics: Clinical trials and innovative delivery systems across diverse diseases. Mol Ther Nucleic Acids 36: 102666, 2025.
131. Hong X, Kim ES and Guo H: Epigenetic regulation of hepatitis B virus covalently closed circular DNA: Implications for epigenetic therapy against chronic hepatitis B. Hepatology 66: 2066-2077, 2017.
132. Ashraf MU, Salman HM, Khalid MF, Khan MHF, Anwar S, Afzal S, Idrees M and Chaudhary SU: CRISPR-Cas13a mediated targeting of hepatitis C virus internal-ribosomal entry site (IRES) as an effective antiviral strategy. Biomed Pharmacother 136: 111239, 2021.
133. Yao ZQ, Schank MB, Zhao J, El Gazzar M, Wang L, Zhang Y, Hill AC, Banik P, Pyburn JS and Moorman JP: The potential of HBV cure: An overview of CRISPR-mediated HBV gene disruption. Front Genome Ed 6: 1467449, 2024.
134. McCoullough LC, Revill P, Littlejohn M (eds): Targeting the HBV pregenomic RNA and viral mRNAs using the CRISPR-Cas13b system and determining the impact of HBV splice variants Sp3 and Sp9 on wildtype HBV replication. University of Melbourne, 2023.
135. Martinez MG, Smekalova E, Combe E, Gregoire F, Zoulim F and Testoni B: Gene editing technologies to target HBV cccDNA. Viruses 14: 2654, 2022.
136. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A and Liu DR: Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576: 149-157, 2019.
137. Carroll D: Genome engineering with zinc-finger nucleases. Genetics 188: 773-782, 2011.
138. Li X, Ding R and Cai J: Organ transplantation: Current status, challenges, and future prospects. MedComm (2020) 7: e70567, 2026.
139. Pauthner M, Yeung J, Ullman C, Bakker J, Wurch T, Reichert JM, Lund-Johansen F, Bradbury AR, Carter PJ and Melis JP: Antibody engineering & therapeutics, the annual meeting of the antibody society December 7-10, 2015, San Diego, CA, USA. MAbs 8: 617-652, 2016.
140. Musallam KM, Bou-Fakhredin R, Cappellini MD and Taher AT: 2021 update on clinical trials in beta-thalassemia. Am J Hematol 96: 1518-1531, 2021.
141. Almotiri A, Abogosh A, Abdelfattah A, Alowaisy D and Rodrigues NP: Treating genetic blood disorders in the era of CRISPR-mediated genome editing. Mol Ther 33: 2645-2662, 2025.
142. Lu Y, Xue J, Deng T, Zhou X, Yu K, Huang M, Liang M, Deng L, Huang J, Ding Z, *et al*: A phase I trial of PD-1 deficient engineered T cells with CRISPR/Cas9 in patients with advanced non-small cell lung cancer. J Clin Oncol 36: 3050, 2018.
143. Hou M, Cheng L, Zhu H, Gui Y and Lei J: Osteopontin promotes lung cancer growth through NF-κB-Dependent upregulation of PD-L1 and suppression of anti-tumor immunity. Mol Biol Rep 53: 1344, 2026.
144. Frangoul H, Locatelli F, Bhatia M, Mapara MY, Molinari L, Sharma A, Lobitz S, de Montalembert M, Rondelli D, Steinberg M, *et al*: Efficacy and safety of a single dose of exagamglogene autotemcel for severe sickle cell disease. Blood 140 (Suppl 1): S29-S31, 2022.
145. Galdikaite-Braziene E, Krušnauskas R, Henderson E and Bujakowska KM: CRISPR as a therapeutic tool for inherited retinal degenerations: Advances, challenges, and future directions. Mol Aspects Med 108: 101462, 2026.
146. Chiu W, Lin TY, Chang YC, Isahwan-Ahmad Mulyadi Lai H, Lin SC, Ma C, Yarmishyn AA, Lin SC, Chang KJ, Chou YB, *et al*: An update on gene therapy for inherited retinal dystrophy: Experience in Leber congenital amaurosis clinical trials. Int J Mol Sci 22: 4534, 2021.
147. Ahmad I: CRISPR/Cas9-a promising therapeutic tool to cure blindness: Current scenario and future prospects. Int J Mol Sci 23: 11482, 2022.
148. Zhou Z, Chen W, Yu H, Tabas I, Foo RS and Tao W: mRNA medicine for cardiovascular disease. Nat Cardiovasc Res 5: 291-307, 2026.
149. Hellenbart EL, Ipema HJ, Rodriguez-Ziccardi MC, Krishna H and DiDomenico RJ: Disease-modifying therapies for amyloid transthyretin cardiomyopathy: Current and emerging medications. Pharmacotherapy 45: 124-144, 2025.
150. Xu F, Zheng C, Xu W, Zhang S, Liu S, Chen X and Yao K: Breaking genetic shackles: The advance of base editing in genetic disorder treatment. Front Pharmacol 15: 1364135, 2024.
151. Wan P, Tang S, Lin D, Lu Y, Long M, Xiao L, Jiang Y, Liao J, Ma X, Liu Y, *et al*: In vivo base editing gene therapy for heterozygous familial hypercholesterolemia: A phase 1 trial. Nat Med 32: 1045-1051, 2026.
152. Khanal S, Schank M, El Gazzar M, Moorman JP and Yao ZQ: HIV-1 latency and viral reservoirs: Existing reversal approaches and potential technologies, targets, and pathways involved in HIV latency studies. Cells 10: 475, 2021.
153. Baylot V, Le TK, Taïeb D, Rocchi P and Colleaux L: Between hope and reality: Treatment of genetic diseases through nucleic acid-based drugs. Commun Biol 7: 489, 2024.
154. Cho E, Lee J, Kim J, Choi J, Kang M and Song J: Application of extracellular vesicles in the CRISPR-based diagnosis and treatment: Possibilities and challenges. J Biol Eng 20: 92, 2026.
155. Kim J, Eygeris Y, Ryals RC, Jozić A and Sahay G: Strategies for non-viral vectors targeting organs beyond the liver. Nat Nanotechnol 19: 428-447, 2024.
156. Zwi-Dantsis L, Mohamed S, Massaro G and Moeendarbary E: Adeno-associated virus vectors: Principles, practices, and prospects in gene therapy. Viruses 17: 239, 2025.
157. Grigg S, Shembrey C, Fareh M, Blombery P, Corn JE, Seymour JF and Casan JML: CRISPR in clinical oncology: Translational advances from molecular diagnostics to therapeutics. Nat Rev Clin Oncol 23: 743-765, 2026.
158. Yudaeva A, Kostyusheva A, Kachanov A, Brezgin S, Ponomareva N, Parodi A, Pokrovsky VS, Lukashev A, Chulanov V and Kostyushev D: Clinical and translational landscape of viral gene therapies. Cells 13: 1916, 2024.
159. Arrasate A, Lopez-Robles C, Zuazo M, Banos-Mateos S, Martin C, Lamsfus-Calle A and Fertin MJ: Lentiviral vectors: from wild-type viruses to efficient multi-functional delivery vectors. Int J Mol Sci 26: 8497, 2025.
160. Zhu P, Xiao L and Wang Y: Mechanisms of high-density lipoprotein in regulating blood-brain barrier function: Insights and implications. Fluids Barriers CNS 22: 113, 2025.
161. Hiranniramol K, Chen Y, Liu W and Wang X: Generalizable sgRNA design for improved CRISPR/Cas9 editing efficiency. Bioinformatics 36: 2684-2689, 2020.
162. Chuai G, Ma H, Yan J, Chen M, Hong N, Xue D, Zhou C, Zhu C, Chen K, Duan B, *et al*: DeepCRISPR: Optimized CRISPR guide RNA design by deep learning. Genome Biol 19: 80, 2018.
163. Baker M: Gene editing at CRISPR speed. Nat Biotechnol 32: 309-313, 2014.
164. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, Li Y, Fine EJ, Wu X, Shalem O, *et al*: DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol 31: 827-832, 2013.
165. Alariqi M, Ramadan M, Yu L, Hui F, Hussain A, Zhou X, Yu Y, Zhang X and Jin S: Enhancing specificity, precision, accessibility, flexibility, and safety to overcome traditional CRISPR/Cas editing challenges and shape future innovations. Adv Sci (Weinh) 12: e2416331, 2025.

166. Tao J, Bauer DE and Chiarle R: Assessing and advancing the safety of CRISPR-Cas tools: from DNA to RNA editing. Nat Commun 14: 212, 2023.
167. Shams F, Bayat H, Mohammadian O, Mahboudi S, Vahidnezhad H, Soosanabadi M and Rahimpour A: Advance trends in targeting homology-directed repair for accurate gene editing: An inclusive review of small molecules and modified CRISPR-Cas9 systems. Bioimpacts 12: 371-391, 2022.
168. Asaad W, Volos P, Maksimov D, Khavina E, Deviatkin A, Mityaeva O and Volchkov P: AAV genome modification for efficient AAV production. Heliyon 9: e15071, 2023.
169. Haider S and Mussolino C: Fine-tuning homology-directed repair (HDR) for precision genome editing: Current strategies and future directions. Int J Mol Sci 26: 4067, 2025.
170. Huang K, Tian J, Zhao W, Zhang S, Xiang L, Yang M, Liu L, Huang Y, Zhang N, Sun L, *et al*: Strategies and mechanisms of precision genome engineering: From gene editing to genome writing. IMetaOmics 3: e70115, 2026.
171. Bollen Y, Post J, Koo BK and Snippert HJG: How to create state-of-the-art genetic model systems: Strategies for optimal CRISPR-mediated genome editing. Nucleic Acids Res 46: 6435-6454, 2018.
172. Madigan V, Zhang F and Dahlman JE: Drug delivery systems for CRISPR-based genome editors. Nat Rev Drug Discov 22: 875-894, 2023.
173. Gil JS, Lee S and Koo T: Therapeutic in vivo genome editing: Innovations and challenges in rAAV vector-based CRISPR delivery. Gene Ther 33: 97-106, 2026.
174. Akagawa R, Nabeshima YI and Kawauchi T: Alternative functions of cell cycle-related and DNA repair proteins in post-mitotic neurons. Front Cell Dev Biol 9: 753175, 2021.
175. Du Rand A, Hunt J, Samson C, Loef E, Malhi C, Meidinger S, Chen CJ, Nutsford A, Taylor J, Dunbar R, *et al*: Highly efficient CRISPR/Cas9-mediated exon skipping for recessive dystrophic epidermolysis bullosa. Bioeng Transl Med 9: e10640, 2024.
176. Yang S, Hu G, Wang J and Song J: CRISPR/Cas-based gene editing tools for large DNA fragment integration. ACS Synth Biol 14: 57-71, 2025.
177. Zhang H, Li Y, Li J, Li X and Li T: Base and prime editing for inherited retinal diseases: Delivery platforms, safety, efficacy, and translational perspectives. Pharmaceutics 17: 1405, 2025.
178. Anzalone AV, Gao XD, Podracky CJ, Nelson AT, Koblan LW, Raguram A, Levy JM, Mercer JAM and Liu DR: Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. Nat Biotechnol 40: 731-740, 2022.
179. Pandey S, Gao XD, Krasnow NA, McElroy A, Tao YA, Duby JE, Steinbeck BJ, McCreary J, Pierce SE, Tolar J, *et al*: Efficient site-specific integration of large genes in mammalian cells via continuously evolved recombinases and prime editing. Nat Biomed Eng 9: 22-39, 2025.
180. Xu Z, Thomas L, Davies B, Chalmers R, Smith M and Brown W: Accuracy and efficiency define Bxb1 integrase as the best of fifteen candidate serine recombinases for the integration of DNA into the human genome. BMC Biotechnol 13: 87, 2013.
181. Lampe GD, King RT, Halpin-Healy TS, Klompe SE, Hogan MI, Vo PLH, Tang S, Chavez A and Sternberg SH: Targeted DNA integration in human cells without double-strand breaks using CRISPR-associated transposases. Nat Biotechnol 42: 87-98, 2024.
182. Haapaniemi E, Botla S, Persson J, Schmierer B and Taipale J: CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med 24: 927-930, 2018.
183. Ihry RJ, Worringer KA, Salick MR, Frias E, Ho D, Theriault K, Kommineni S, Chen J, Sondey M, Ye C, *et al*: p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med 24: 939-946, 2018.
184. Enache OM, Rendo V, Abdusamad M, Lam D, Davison D, Pal S, Currimjee N, Hess J, Pantel S, Nag A, *et al*: Cas9 activates the p53 pathway and selects for p53-inactivating mutations. Nat Genet 52: 662-668, 2020.
185. Hanlon KS, Kleinstiver BP, Garcia SP, Zaborowski MP, Volak A, Spirig SE, Muller A, Sousa AA, Tsai SQ, Bengtsson NE, *et al*: High levels of AAV vector integration into CRISPR-induced DNA breaks. Nat Commun 10: 4439, 2019.
186. Bazick HO, Mao H, Niehaus JK, Wolter JM and Zylka MJ: AAV vector-derived elements integrate into Cas9-generated double-strand breaks and disrupt gene transcription. Mol Ther 32: 4122-4137, 2024.
187. Charlesworth CT, Deshpande PS, Dever DP, Camarena J, Lemgart VT, Cromer MK, Vakulskas CA, Collingwood MA, Zhang L, Bode NM, *et al*: Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat Med 25: 249-254, 2019.
188. Workenhe ST, Pol J and Kroemer G: Tumor-intrinsic determinants of immunogenic cell death modalities. Oncoimmunology 10: 1893466, 2021.
189. Li A, Tanner MR, Lee CM, Hurley AE, De Giorgi M, Jarrett KE, Davis TH, Doerfler AM, Bao G, Beeton C and Lagor WR: AAV-CRISPR gene editing is negated by pre-existing immunity to Cas9. Mol Ther 28: 1432-1441, 2020.
190. Ewaisha R and Anderson KS: Immunogenicity of CRISPR therapeutics-Critical considerations for clinical translation. Front Bioeng Biotechnol 11: 1138596, 2023.
191. Anzalone AV, Koblan LW and Liu DR: Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. Nat Biotechnol 38: 824-844, 2020.
192. Raghavan R, Friedrich MJ, King I, Chau-Duy-Tam Vo S, Strebinger D, Lash B, Kilian M, Platten M, Macrae RK, Song Y, *et al*: Rational engineering of minimally immunogenic nucleases for gene therapy. Nat Commun 16: 105. 2025.
193. Wilbie D, Walther J and Mastrobattista E: Delivery aspects of CRISPR/Cas for in vivo genome editing. Acc Chem Res 52: 1555-1564, 2019.
194. Mehravar M, Shirazi A, Nazari M and Banan M: Mosaicism in CRISPR/Cas9-mediated genome editing. Dev Biol 445: 156-162, 2019.
195. Cutillas-Tolín A, Adoamnei E, Navarrete-Muñoz EM, Vioque J, Moñino-García M, Jørgensen N, Chavarro JE, Mendiola J and Torres-Cantero AM: Adherence to diet quality indices in relation to semen quality and reproductive hormones in young men. Hum Reprod 34: 1866-1875, 2019.
196. Bao C, Channell CI, Tseng YH, Bailey J, Sbaiti N, Demirkol A and Tsang SH: Chronic in vivo CRISPR-Cas genome editing: Challenges, long-term safety, and outlook. Cells 15: 156, 2026.
197. Youssef E, Weddle K, Zimmerman L and Palmer D: Pharmacovigilance in cell and gene therapy: Evolving challenges in risk management and long-term follow-Up. Drug Saf 49: 27-53, 2026.
198. Khare G, Duong A, Hanotaux J, Shorr R, Nampoothiri RV, Maganti H and Allan DS: A scoping review of gene editing in clinical trials: identifying aspects of trial design to accelerate clinical adoption. Cytotherapy 28: 102898, 2026.
199. Bashir K, Vasudev P, Chhetri V, Dubhashi DP, Teli YG, Biswas R, Ramaiah S and Anbarasu A: Clinical translation of CRISPR-Cas9 therapeutics in cancer and inherited genetic disorders. Front Genome Ed 8:1868779, 2026.
200. Chen R, Zhou H, Wu Q. and Gong C: Nano delivery systems for in vivo CRISPR/Cas-Based gene therapy: From vectors to clinical outcomes. ACS Nano 20: 27559-27640, 2026.
201. FDA: Long Term Follow-up After Administration of Human Gene Therapy Products. Center for Biologics Evaluation and Research. Guidance for Industry, 2020.
202. Tay LS, Palmer N, Panwala R, Chew WL and Mali P: Translating CRISPR-Cas therapeutics: Approaches and challenges. CRISPR J 3: 253-275, 2020.
203. Van Beers BC: Rewriting the human genome, rewriting human rights law? Human rights, human dignity, and human germline modification in the CRISPR era. J Law Biosci 7: lsaa006, 2020.
204. Tunis SR, Clarke M, Gorst SL, Gargon E, Blazeby JM, Altman DG and Williamson PR: Improving the relevance and consistency of outcomes in comparative effectiveness research. J Comp Eff Res 5: 193-205, 2016.
205. Seijas A, Cora D, Novo M, Al-Soufi W, Sánchez L and Arana ÁJ: CRISPR/Cas9 delivery systems to enhance gene editing efficiency. Int J Mol Sci 26: 4420, 2025.
206. Matsumoto D, Matsugi E, Kishi K, Inoue Y, Nigorikawa K and Nomura W: SpCas9-HF1 enhances accuracy of cell cycle-dependent genome editing by increasing HDR efficiency, and by reducing off-target effects and indel rates. Mol Ther Nucleic Acids 35: 102124, 2024.
207. Salman A, Kantor A, McClements ME, Marfany G, Trigueros S and MacLaren RE: Non-viral delivery of CRISPR/Cas cargo to the retina using nanoparticles: Current possibilities, challenges, and limitations. Pharmaceutics 14: 1842, 2022.
208. Chelvaretnam S, Mom KT, Palma Henriquez CA, Pham Q, Fitri A, Bhuiyan S, Shojaee M, Asari K, Rashid H, Hinch L, *et al*: Harnessing CAR-Extracellular vesicles for next-generation cancer immunotherapy. Int J Mol Sci 27: 2163, 2026.

209. Paulusma CC and Bosma PJ: Therapeutic base editing in the adult liver. Nat Rev Gastroenterol Hepatol 18: 597-598, 2021.
210. Wang JY and Doudna JA: CRISPR technology: A decade of genome editing is only the beginning. Science 379: eadd8643, 2023.
211. Rothgangl T, Dennis MK, Lin PJ, Oka R, Witzigmann D, Villiger L, Qi W, Hruzova M, Kissling L, Lenggenhager D, *et al*: In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol levels. Nat Biotechnol 39: 949-957, 2021.
212. Fu L, Zhang Y, Farokhzad RA, Mendes BB, Conde J and Shi J: 'Passive'nanoparticles for organ-selective systemic delivery: Design, mechanism and perspective. Chem Soc Rev 52: 7579-7601, 2023.
213. Wu J, Hua Y, Zheng Y, Liu X and Li Y: Engineering targeted gene delivery systems for primary hereditary skeletal myopathies: Current strategies and future perspectives. Biomedicines 13: 1994, 2025.
214. Bondy-Denomy J: Protein inhibitors of CRISPR-Cas9. ACS Chem Biol 13: 417-423, 2018.
215. Singh K, Jain D, Sethi P, Gupta JK, Dubey A, Al Noman A, Lal S, Sharma PD and Abdallah EM: Advances in viral vector-based delivery systems for gene therapy: A comprehensive review. 3 Biotech 15: 196, 2025.
216. Pawluk A, Davidson AR and Maxwell KL: Anti-CRISPR: Discovery, mechanism and function. Nat Rev Microbiol 16: 12-17, 2018.
217. Stanley SY and Maxwell KL: Phage-encoded anti-CRISPR defenses. Annu Rev Genet 52: 445-464, 2018.
218. Pawluk A, Shah M, Mejdani M, Calmettes C, Moraes TF, Davidson AR and Maxwell KL: Disabling a type IE CRISPR-Cas nuclease with a bacteriophage-encoded anti-CRISPR protein. Mbio 8: e01751-17, 2017.
219. Nonnenmacher M, Wang W, Child MA, Ren XQ, Huang C, Ren AZ, Tocci J, Chen Q, Bittner K, Tyson K, *et al*: Rapid evolution of blood-brain-barrier-penetrating AAV capsids by RNA-driven biopanning. Mol Ther Methods Clin Dev 20: 366-378, 2020.
220. Rauch BJ, Silvis MR, Hultquist JF, Waters CS, McGregor MJ, Krogan NJ and Bondy-Denomy J: Inhibition of CRISPR-Cas9 with bacteriophage proteins. Cell 168: 150-158.e10, 2017.
221. Yang H and Patel DJ: Inhibition mechanism of an anti-CRISPR suppressor AcrIIA4 targeting SpyCas9. Mol Cell 67: 117-127.e5, 2017.
222. Harrington LB, Doxzen KW, Ma E, Liu JJ, Knott GJ, Edraki A, Garcia B, Amrani N, Chen JS, Cofsky JC, *et al*: A broad-spectrum inhibitor of CRISPR-Cas9. Cell 170: 1224-1233,e15, 2017.
223. Liu H, Zhu Y, Lu Z and Huang Z: Structural basis of Staphylococcus aureus Cas9 inhibition by AcrIIA14. Nucleic Acids Res 49: 6587-6595, 2021.
224. Chan KY, Jang MJ, Yoo BB, Greenbaum A, Ravi N, Wu WL, Sánchez-Guardado L, Lois C, Mazmanian SK, Deverman BE and Gradinaru V: Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. Nat Neurosci 20: 1172-1179, 2017.
225. Allemailem KS, Almatroodi SA, Almatroudi A, Alrumaihi F, Al Abdulmonem W, Al-Megrin WAI, Aljamaan AN, Rahmani AH and Khan AA: Recent advances in genome-editing technology with CRISPR/Cas9 variants and stimuli-responsive targeting approaches within tumor cells: A future perspective of cancer management. Int J Mol Sci 24: 7052, 2023.
226. Wiegand T, Karambelkar S, Bondy-Denomy J and Wiedenheft B: Structures and strategies of anti-CRISPR-mediated immune suppression. Annu Rev Microbiol 74: 21-37, 2020.
227. Jia N and Patel DJ: Structure-based functional mechanisms and biotechnology applications of anti-CRISPR proteins. Nat Rev Mol Cell Biol 22: 563-579, 2021.
228. Rehman SU and Abbas GH: CRISPR/CAS9-based gene editing in cancer therapy: A systematic review and meta-analysis on current status and future directions. Medicine (Baltimore) 105: e47114, 2026.
229. Calvet L, Dos-Santos O, Spanakis E, Jean-Baptiste V, Le Bail JC, Buzy A, Paul P, Henry C, Valence S, Dib C, *et al*: YAP1 is essential for malignant mesothelioma tumor maintenance. BMC Cancer 22: 639, 2022.
230. Sun JG, Chen XW, Zhang LP, Wang J and Diehn M: Yap1 promotes the survival and self-renewal of breast tumor initiating cells via inhibiting Smad3 signaling. Oncotarget 7: 9692-9706, 2016.
231. Tsai FJ, Cheng CF, Lai CH, Wu YC, Ho MW, Wang JH, Tien N, Liu X, Tsang H, Lin TH, *et al*: Effect of antiretroviral therapy use and adherence on the risk of hyperlipidemia among HIV-infected patients, in the highly active antiretroviral therapy era. Oncotarget 8: 106369-106381, 2017.
232. Wang P, Liu Z, Zhang X, Li J, Sun L, Ju Z, Li J, Chan P, Liu GH, Zhang W, *et al*: CRISPR/Cas9-mediated gene knockout reveals a guardian role of NF-κB/RelA in maintaining the homeostasis of human vascular cells. Protein Cell 9: 945-965, 2018.
233. Hoffmann A, Natoli G and Ghosh G: Transcriptional regulation via the NF-kappaB signaling module. Oncogene 25: 6706-6716, 2006.
234. Sayed S, Sidorova OA, Hennig A, Augsburg M, Cortés Vesga CP, Abohawya M, Schmitt LT, Sürün D, Stange DE, Mircetic J and Buchholz F: Efficient correction of oncogenic KRAS and TP53 mutations through CRISPR base editing. Cancer Res 82: 3002-3015, 2022.
235. Wang Z, Burigotto M, Ghetti S, Vaillant F, Tan T, Capaldo BD, Palmieri M, Hirokawa Y, Tai L, Simpson DS, *et al*: Loss-of-function but not gain-of-function properties of mutant TP53 are critical for the proliferation, survival, and metastasis of a broad range of cancer cells. Cancer Discov 14: 362-379, 2024.
236. Chira S, Gulei D, Hajitou A and Berindan-Neagoe I: Restoring the p53 'Guardian'phenotype in p53-deficient tumor cells with CRISPR/Cas9. Trends Biotechnol 36: 653-660, 2018.
237. Tsujino T, Takai T, Hinohara K, Gui F, Tsutsumi T, Bai X, Miao C, Feng C, Gui B, Sztupinszki Z, *et al*: CRISPR screens reveal genetic determinants of PARP inhibitor sensitivity and resistance in prostate cancer. Nat Commun 14: 252, 2023.
238. Dubrot J, Du PP, Lane-Reticker SK, Kessler EA, Muscato AJ, Mehta A, Freeman SS, Allen PM, Olander KE, Ockerman KM, *et al*: In vivo CRISPR screens reveal the landscape of immune evasion pathways across cancer. Nat Immunol 23: 1495-1506, 2022.
239. Wang S, Iyer R, Han X, Wei J, Li N, Cheng Y, Zhou Y, Gao Q, Zhang L, Yan M, *et al*: CRISPR screening identifies the deubiquitylase ATXN3 as a PD-L1-positive regulator for tumor immune evasion. J Clin Invest 133: e167728, 2023.
240. Li B, Wei P, Xie B, Zhong X, Wang X, Lou X, Zhang T and Dong C: Targeting tumor-intrinsic CDK1/Cyclin B1 complex improves responses to immunotherapy in pancreatic cancer. Cancer Lett 648: 218464, 2026.
241. Lu Y, Bouchard C, Soucy N, Siddika A, Lamothe G, Godbout K and Tremblay JP: The improvements and applications of prime editing. DNA 6: 16, 2026.
242. Fayed S, Amer S, Badawy M, Bou Malhab L, Omran N, Khoder G, Ghemrawi R, Haider M, Hamoudi R and Harati R: The role of CRISPR and its therapeutic applications in glioblastoma. Int J Mol Sci 27: 2008, 2026.
243. Shi S, Ou X, Liu C, Li R, Zheng Q and Hu L: NF-κB signaling and the tumor microenvironment in osteosarcoma: Implications for immune evasion and therapeutic resistance. Front Immunol 16: 1518664, 2025.
244. Wen J, Fu L, Zhong H and Chen H: The role of P53 in immune evasion and therapeutic strategies in hematologic malignancies. J Cancer 16: 3899-3906, 2025.
245. Xiao Y, Chen J, Zhou H, Zeng X, Ruan Z, Pu Z, Jiang X, Matsui A, Zhu L, Amoozgar Z, *et al*: Combining p53 mRNA nanotherapy with immune checkpoint blockade reprograms the immune microenvironment for effective cancer therapy. Nat Commun 13: 758, 2022.
246. Okada M, Kanamori M, Someya K, Nakatsukasa H and Yoshimura A: Stabilization of Foxp3 expression by CRISPR-dCas9-based epigenome editing in mouse primary T cells. Epigenetics Chromatin 10: 24, 2017.
247. Doench JG, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, Smith I, Tothova Z, Wilen C, Orchard R, *et al*: Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat Biotechnol 34: 184-191, 2016.
248. Cancellieri S, Canver MC, Bombieri N, Giugno R and Pinello L: CRISPRitz: Rapid, high-throughput and variant-aware in silico off-target site identification for CRISPR genome editing. Bioinformatics 36: 2001-2008, 2020.
249. Yuan H, Song C, Xu H, Sun Y, Anthon C, Bolund L, Lin L, Benabdellah K, Lee C, Hou Y, *et al*: An overview and comparative analysis of CRISPR-SpCas9 gRNA activity prediction tools. CRISPR J 8: 89-104, 2025.
250. Cao M, Brennan A, Lee CM, Park SH and Bao G: Deep learning based models for CRISPR/Cas off-target prediction. Small Methods 9: e2500122, 2025.

251. Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, Gill S, Harrington WF, Pantel S, Krill-Burger JM, *et al*: Defining a cancer dependency map. Cell 170: 564-576.e16, 2017.
252. Du W, Zhang T, Guo L, Zheng Y, Zhang H, Zhu X, Tang D, Hu H, Chen L and Liu C: Deep learning-driven prediction of on-target activity, off-target risk, and repair outcomes in CRISPR/Cas9: Current landscape and multi-scale perspectives. J Transl Med 24: 769, 2026.
253. Thomson T, Li G, Strilchuk A, Cui H, Wang B and Li B: Harnessing artificial intelligence to advance CRISPR-based genome editing technologies. Nat Rev Genet 27: 212-230, 2026.

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