

## CRISPR-CAS GENOME EDITING IN PHARMACEUTICAL BIOTECHNOLOGY: ADVANCES, THERAPEUTIC APPLICATIONS, CHALLENGES AND FUTURE PERSPECTIVES

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### ABSTRACT

CRISPR-Cas genome editing has evolved from a bacterial adaptive immune mechanism into a validated pharmaceutical platform spanning drug discovery, direct genetic therapeutics, and molecular diagnostics. This review provides a pharmaceutically oriented synthesis of the technology's mechanistic foundations, its evolution from first-generation Cas9 nucleases through base editing, prime editing, and CRISPR interference/activation, and its applications across the drug discovery and development pipeline, including target identification, functional genomic screening, and disease modeling. We examine the therapeutic landscape in detail, covering oncology, hematological disorders, infectious disease, and metabolic and neurological conditions, alongside the ex vivo and in vivo gene- and cell-therapy platforms that have brought these approaches to the clinic, exemplified by the regulatory approval of Casgevy and the growing pipeline of in vivo hepatic editing programs. Particular attention is given to the delivery technologies such as viral, non-viral, and lipid-nanoparticle-based that increasingly represent the rate-limiting step in clinical translation, and to CRISPR-based diagnostics and their convergence with precision medicine. This review then addresses the principal challenges constraining pharmaceutical translation, including off-target effects, immunogenicity, genome stability, and manufacturing scalability, together with the ethical and regulatory considerations particularly the somatic-germline distinction and equitable access that shape how these technologies are deployed. We close with an assessment of emerging trends, including artificial-intelligence-assisted guide RNA design and next-generation delivery platforms, prospects for clinical translation, and a discussion of the limitations inherent in synthesizing a rapidly evolving field. Collectively, this review aims to provide researchers and drug developers with a comprehensive, integrated view of CRISPR-Cas technology's current state and trajectory within pharmaceutical biotechnology.

**KEYWORDS:** CRISPR-Cas9; genome editing; base editing; prime editing; gene therapy; pharmaceutical biotechnology; drug discovery; precision medicine.

## 1. INTRODUCTION

### 1.1. Background of Genome Editing

Genome editing refers to a set of molecular tools that allow researchers to make precise, targeted changes to an organism's DNA including inserting, deleting, or replacing genetic sequences at defined locations rather than relying on random mutagenesis. This capacity to rewrite the genetic code with locus-level precision has transformed biomedical research over the past two decades, providing the means to interrogate gene function directly, generate disease-relevant cellular and animal models, and, more recently, to correct disease-causing mutations in patients.<sup>[1,2]</sup>

Before the CRISPR era, two engineered nuclease platforms dominated the field: zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). ZFNs combine arrays of Cys2-His2 zinc-finger DNA-binding domains, each recognizing roughly three base pairs, with the non-specific cleavage domain of the FokI endonuclease; dimerization of two ZFN monomers at adjacent sites generates a double-strand break that stimulates cellular repair pathways.<sup>[3,4]</sup> TALENs use a related strategy, substituting the zinc-finger domains for transcription activator-like effector (TALE) repeat arrays derived from *Xanthomonas* plant pathogens, in which each repeat recognizes a single base pair via a variable diresidue<sup>[4,5]</sup>. Both platforms demonstrated that programmable, site-specific genome editing was achievable in living cells and animals, and both were used clinically and pre-clinically before CRISPR's emergence.<sup>[3]</sup>

However, ZFNs and TALENs share a fundamental limitation: target specificity is encoded in a large, custom-engineered protein, so every new genomic target requires laborious protein design, assembly, and validation.<sup>[3,5]</sup> This made ZFN and TALEN platforms expensive, slow to redesign, and inaccessible to most academic laboratories, constraining both the pace of functional genomics research and the scalability of therapeutic development. It was this combination of high cost, technical difficulty, and limited throughput that created the opening for a fundamentally different, RNA-guided approach to genome targeting.<sup>[3,5]</sup>

### 1.2. Emergence of CRISPR-Cas Technology

CRISPR (clustered regularly interspaced short palindromic repeats) was first identified not as a genome-editing tool but as a naturally occurring adaptive immune system in bacteria and archaea, used to recognize and destroy invading bacteriophage and plasmid DNA<sup>[4,6]</sup>. Bacteria capture short fragments of invading genetic material and store them as "spacers" between repeat sequences in their own genome; upon re-exposure to the same invader, these spacers are transcribed into short guide RNAs that direct a CRISPR-associated (Cas) nuclease to cleave the matching foreign sequence.<sup>[4,6]</sup>

The transformative insight, credited principally to the work of Jennifer Doudna, Emmanuelle Charpentier, and collaborators, was that this bacterial immune mechanism could be repurposed as a programmable gene-editing tool: a single synthetic guide RNA could direct the Cas9 nuclease to cleave virtually any DNA sequence complementary to it, provided a short protospacer-adjacent motif (PAM) was present.<sup>[4,6]</sup> Because target specificity is now dictated by a short, easily synthesized RNA sequence rather than an engineered protein, redesigning CRISPR-Cas9 for a new genomic target requires only the design of a new guide RNA a process that can be completed in days rather than the weeks-to-months needed for ZFN or TALEN engineering.<sup>[3,5]</sup>

This simplicity, combined with high efficiency, low cost, and the ability to multiplex several guide RNAs simultaneously, is what allowed CRISPR-Cas9 to eclipse earlier platforms within a few years of its introduction and to be adopted rapidly across essentially every subfield of biomedical and pharmaceutical research.<sup>[2,3]</sup>

### 1.3. Relevance of CRISPR to Pharmaceutical Biotechnology

CRISPR's impact on pharmaceutical biotechnology extends well beyond its use as a laboratory research tool. Pooled CRISPR knockout and activation screens now allow systematic, genome-wide interrogation of gene function in disease-relevant cell models, enabling unbiased identification and validation of novel drug targets at a scale and speed that earlier RNA interference-based approaches could not match.<sup>[7,8,9]</sup> These functional genomic screens have been used to map genes required for cancer cell survival, to identify mediators of drug resistance, and to dissect mechanisms of drug action directly in cellular systems.<sup>[7,9]</sup>

CRISPR-engineered cell lines and animal models also provide substantially more accurate disease models than earlier approaches, since specific patient-derived mutations can be introduced or corrected in isogenic backgrounds, isolating the phenotypic contribution of a single genetic variant.<sup>[1,10]</sup> This has accelerated both basic disease-mechanism research and the preclinical evaluation of candidate therapeutics.

Most consequentially for the pharmaceutical industry, CRISPR-Cas systems have moved from a research tool into a therapeutic modality in their own right. Ex vivo CRISPR-edited cell therapies, exemplified by the FDA- and MHRA-approved product Casgevy for sickle cell disease and transfusion-dependent  $\beta$ -thalassemia, and in vivo CRISPR therapeutics delivered directly to patients, such as NTLA-2001/nexiguran ziclumeran for transthyretin amyloidosis and NTLA-2002 for hereditary angioedema, demonstrate that genome editing can now be administered as a one-time treatment with durable clinical effect.<sup>[11,12,13,14]</sup> This convergence of CRISPR with drug discovery, cell and gene therapy, and precision medicine is what positions it as one of the defining technology platforms in contemporary pharmaceutical biotechnology.<sup>[2,9]</sup>

### 1.4. Recent Advances and Need for Next-Generation CRISPR Technologies

The original CRISPR-Cas9 system, while transformative, relies on generating a double-strand DNA break, which can produce unpredictable repair outcomes, including large deletions, insertions, and chromosomal rearrangements.<sup>[2,15]</sup> This limitation has driven the rapid development of an expanded CRISPR toolbox. New Cas variants and orthologs including smaller, more easily delivered nucleases such as Cas12a and various compact Cas9 orthologs, as well as catalytically modified nickases have broadened the range of editable targets and improved the safety profile of genome editing.<sup>[16,17]</sup>

Base editing, which fuses a catalytically impaired Cas9 nickase to a deaminase enzyme, enables direct chemical conversion of one DNA base to another without creating a double-strand break, substantially reducing the risk of large unintended genomic rearrangements.<sup>[2,18]</sup> Prime editing extends this principle further, using a Cas9 nickase fused to a reverse transcriptase and a prime-editing guide RNA (pegRNA) to "search and replace" genetic sequences, enabling precise insertions, deletions, and all twelve possible base-to-base conversions without requiring a double-strand break or a donor DNA template.<sup>[15,19,20]</sup>

In parallel, catalytically dead Cas9 (dCas9) fused to transcriptional effector domains has given rise to CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), which allow reversible, tunable modulation of gene expression without altering the underlying DNA sequence, a capability with particular value for functional genomic screening and for therapeutic gene-dosage correction.<sup>[21,22]</sup> Alongside these editor-level innovations, delivery technology has advanced substantially, most notably through ionizable lipid nanoparticles (LNPs) and improved adeno-associated virus (AAV) vector engineering, both of which are now central to translating CRISPR editors from cultured cells into patients.<sup>[23,24]</sup> Collectively, these advances reflect a clear trajectory: the field is moving from laboratory-scale gene disruption toward precise, programmable, and increasingly safe genome and epigenome editing suitable for clinical use.<sup>[16,17]</sup>

### 1.5. Challenges in Pharmaceutical and Clinical Translation

Despite this progress, several substantial challenges continue to constrain the clinical and commercial translation of CRISPR-based technologies. Off-target editing is unintended modification of genomic sequences similar, but not identical, to the intended target that remains a central safety concern, and a range of computational prediction tools and experimental detection methods (including GUIDE-seq, CIRCLE-seq, and Digenome-seq-type assays) have been developed to characterize and minimize this risk.<sup>[25,26,27]</sup>

Delivery remains a persistent bottleneck: achieving efficient, tissue-specific delivery of CRISPR components to the intended organ or cell type, while avoiding hepatic sequestration and minimizing off-target tissue exposure, is technically demanding for both viral and non-viral systems.<sup>[23,24]</sup> Immunogenicity is a related concern, since Cas proteins derived from bacterial sources, as well as the viral or lipid-based delivery vehicles themselves, can provoke innate and adaptive immune responses that compromise efficacy or safety<sup>[16]</sup>. Longer-term questions about genome stability including the potential for large structural variants, chromothripsis, or unintended effects on tumor-suppressor pathways such as p53 activation following double-strand breaks remain an active area of investigation, particularly for therapies intended to be permanent, single-administration treatments.<sup>[15,25]</sup>

Manufacturing and scalability present a further obstacle: many current CRISPR-based cell therapies rely on complex, individualized (autologous) manufacturing processes that are costly and difficult to scale, while viral vector production for in vivo therapies faces its own capacity and cost constraints.<sup>[16,28,29]</sup> Finally, ethical and regulatory questions particularly concerning the distinction between somatic and germline editing, and the pricing and equitable accessibility of multi-million-dollar one-time gene-editing therapies continue to shape how, and how quickly, these technologies can be translated into routine clinical use.<sup>[30,31,32]</sup> Each of these challenges is addressed in greater depth in Sections 8 and 9 of this review.

### 1.6. Aim and Scope of the Review

This review provides a comprehensive, pharmaceutically oriented synthesis of CRISPR-Cas genome-editing technology, spanning its mechanistic foundations, its applications across the drug discovery and development pipeline, its use as a direct therapeutic modality, and the delivery, safety, manufacturing, ethical, and regulatory considerations that govern its clinical translation. The review further considers CRISPR-based diagnostics and their convergence with precision medicine, and closes with an assessment of emerging technologies including base and prime editing, artificial-intelligence-assisted guide RNA design, and next-generation delivery platforms that are likely to shape the next phase of CRISPR-enabled drug discovery and therapeutic development.

## 2. CRISPR-Cas Genome Editing: Evolution and Technological Advancements

### 2.1. Discovery and Development of CRISPR-Cas Systems

CRISPR loci were first observed in bacterial genomes in the late 1980s, long before their biological function was understood, and their role as a sequence-specific adaptive immune system against bacteriophages and mobile genetic elements was established through subsequent microbiological research over the following two decades.<sup>[4,6]</sup> The identification of the type II CRISPR-Cas9 system from *Streptococcus pyogenes*, and the demonstration that a single engineered guide RNA could direct Cas9 to introduce a site-specific double-strand break in DNA in vitro, provided the conceptual and mechanistic basis for repurposing CRISPR as a programmable genome-editing tool.<sup>[4,6]</sup> This was rapidly followed by demonstrations that CRISPR-Cas9 could function in mammalian cells, cementing its status as a general-purpose genome-editing platform applicable across essentially all model organisms and cell types.<sup>[2,4]</sup>

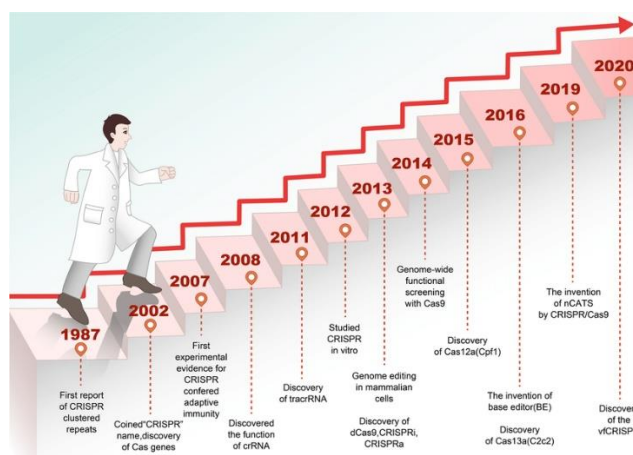


Figure 1: Evolution of CRISPR-Cas Genome Editing Technologies.

### 2.2. CRISPR-Cas9 Genome Editing Mechanism

The canonical CRISPR-Cas9 system requires three core components: the Cas9 endonuclease, a CRISPR RNA (crRNA) encoding the target-recognition sequence, and a trans-activating crRNA (tracrRNA) that facilitates crRNA processing and Cas9 loading; in engineered systems, the crRNA and tracrRNA are typically fused into a single synthetic guide RNA (sgRNA).<sup>[4,6]</sup> The Cas9-sgRNA ribonucleoprotein complex scans genomic DNA for a short protospacer-adjacent motif (PAM, canonically 5'-NGG-3' for *S. pyogenes* Cas9); upon PAM recognition, the adjacent DNA is unwound, and the sgRNA spacer sequence forms an RNA-DNA heteroduplex with the complementary genomic strand.<sup>[4]</sup> Correct base-pairing across the guide sequence triggers a conformational change that activates the two nuclease domains of Cas9-RuvC and HNH which together cleave both DNA strands roughly three base pairs upstream of the PAM, generating a blunt-ended double-strand break.<sup>[4,6]</sup>

This break is then repaired by one of two endogenous cellular pathways. Non-homologous end joining (NHEJ), the dominant pathway in most cell types, directly rejoins the cleaved ends and frequently introduces small insertions or deletions (indels) at the cut site, which can disrupt gene function through frameshift mutations, the basis of CRISPR-mediated gene knockout.<sup>[4]</sup> Alternatively, in the presence of a homologous DNA template, the cell may repair the break via homology-directed repair (HDR), enabling precise insertion or correction of a defined sequence, though HDR is generally less efficient than NHEJ and is largely restricted to dividing cells.<sup>[4]</sup>

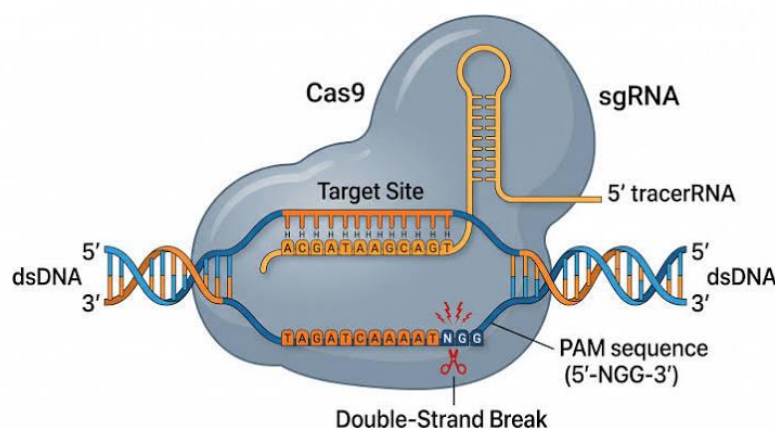


**Figure 2: Mechanism of CRISPR-Cas9-Mediated Genome Editing.**

### 2.3. Evolution of CRISPR-Cas Systems

Since the original description of Cas9, the CRISPR toolbox has expanded substantially, with several hundred distinct CRISPR-Cas systems now characterized across diverse bacterial and archaeal species.<sup>[16]</sup> Cas12a (formerly Cpf1) emerged as a widely adopted alternative to Cas9, offering a smaller protein size that facilitates viral packaging, a distinct T-rich PAM that expands the range of targetable sequences, and staggered rather than blunt double-strand-break ends.<sup>[16,17]</sup> Cas12a has since been used clinically, including in CRISPR-Cas12a edited cell therapies for sickle cell disease and  $\beta$ -thalassemia that disrupt the *HBG1/HBG2* promoters to reactivate fetal hemoglobin production.<sup>[33]</sup>

Cas13 systems, which target RNA rather than DNA, extended CRISPR's utility beyond permanent genomic editing into transient RNA-level modulation and, notably, into molecular diagnostics: Cas13's collateral trans-cleavage activity upon target recognition underlies CRISPR-based diagnostic platforms such as SHERLOCK, while the related Cas12-based DETECTR platform exploits analogous collateral single-stranded DNA cleavage.<sup>[34,35]</sup> Engineering efforts have also produced smaller, more clinically tractable Cas orthologs and catalytically modified variants including nickases that cleave only one DNA strand and catalytically "dead" Cas9 (dCas9) that retains DNA-binding but not cleavage activity which together have enabled the development of base editors, prime editors, and CRISPR-based transcriptional regulation tools described below.<sup>[16,21]</sup>

### 2.4. Next-Generation CRISPR Technologies

Base editors couple a Cas9 nickase to a deaminase enzyme called cytosine base editors (CBEs) that convert C•G to T•A base pairs, while adenine base editors (ABEs) convert A•T to G•C, allowing single-nucleotide substitutions to be installed without generating a double-strand break, thereby avoiding the large-scale genomic rearrangements associated with canonical Cas9-mediated cleavage.<sup>[2,18]</sup> Base editing has already entered clinical evaluation, including in vivo base editing of *PCSK9* to durably lower cholesterol and, more recently, base editing of the *HBG1/HBG2* promoters for sickle cell disease.<sup>[36,37]</sup>

Prime editing represents a further mechanistic advance, using a Cas9 nickase fused to an engineered reverse transcriptase, directed by a prime-editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit. This "search-and-replace" mechanism enables precise insertions, deletions, and all twelve possible base-to-base conversions without requiring a double-strand break or an exogenous donor DNA template, and successive

generations of prime editors (PE2 through PE6 and beyond) have progressively improved editing efficiency and expanded the size of insertions and deletions that can be achieved.<sup>[15,19,20]</sup>

CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) use catalytically dead Cas9 fused to repressive (e.g., KRAB) or activating (e.g., VP64, VPR, p300) effector domains, respectively, to modulate target gene transcription without permanently altering the DNA sequence.<sup>[21,22]</sup> Because these effects are reversible and tunable, CRISPRi/a platforms are particularly valuable for functional genomic screening and are being explored therapeutically, including in preclinical models of neurodegenerative disease.<sup>[21]</sup> Finally, artificial intelligence and deep learning are increasingly being integrated into CRISPR tool development itself, with machine learning models now used to predict guide RNA on-target efficiency and off-target risk, reflecting a broader trend toward computationally guided design of next-generation genome-editing reagents.<sup>[38,39]</sup> These next-generation platforms are base editing, prime editing, CRISPRi/a, and AI-assisted design that are revisited in greater depth in Section 10 in the context of their emerging clinical and drug-discovery applications.

**Table 1: Comparison of major CRISPR-based genome-editing and gene-regulation technologies.**

| Technology    | Target                    | Mechanism                  | Major outcome                     | Major advantage               | Major limitation              |
|---------------|---------------------------|----------------------------|-----------------------------------|-------------------------------|-------------------------------|
| Cas9          | DNA                       | DSB                        | Knockout/knock-in                 | Versatile                     | DSB-associated risks          |
| Cas12a        | DNA                       | DSB                        | Gene editing                      | Different PAM/compact options | Target constraints            |
| Cas13         | RNA                       | RNA cleavage               | RNA targeting/editing/diagnostics | No permanent DNA alteration   | RNA-level effects             |
| Base editing  | DNA                       | Base conversion            | Point mutation correction         | No DSB                        | Editing window/by-products    |
| Prime editing | DNA                       | Reverse transcription      | Substitution/insertion/deletion   | Broad editing capability      | Efficiency/delivery           |
| CRISPRi       | DNA-associated regulation | Transcriptional repression | Gene knockdown                    | Reversible                    | Requires sustained expression |
| CRISPRa       | DNA-associated regulation | Transcriptional activation | Gene activation                   | Tunable                       | Delivery/complexity           |

### 3. CRISPR in Pharmaceutical Drug Discovery and Development

#### 3.1. CRISPR-Based Target Identification and Validation

One of CRISPR's most direct contributions to pharmaceutical research has been in target identification and validation. Pooled CRISPR knockout libraries, typically delivered by lentiviral transduction, allow researchers to systematically disrupt every gene in the genome across a population of cells and then identify, through guide RNA sequencing, which genes are required for a given phenotype most commonly cell survival, proliferation, or resistance to a specific drug.<sup>[7,9]</sup> Because this approach is unbiased and genome-wide, it avoids the need to hypothesize a candidate target in advance, and has proven particularly powerful for identifying genes whose loss selectively sensitizes disease cells (for example, cancer cells bearing a specific oncogenic driver) while sparing normal cells a key criterion for a viable drug target.<sup>[7,40]</sup>

CRISPR-based target deconvolution has also been applied directly to small-molecule pharmacology: by linking cellular resistance or sensitivity to a compound with CRISPR-mediated gene perturbation, researchers can identify the protein(s) a small molecule acts upon without needing to chemically modify the compound itself, complementing

traditional affinity-based target identification methods.<sup>[9]</sup> This has made CRISPR screening one of the standard early-stage tools in pharmaceutical target discovery pipelines.<sup>[7,8]</sup>

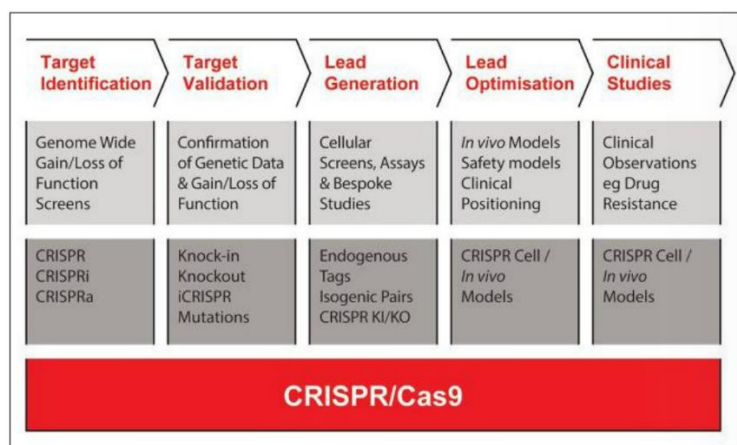


**Figure 3: Applications of CRISPR-Cas9 in the Drug Discovery Process.**

### 3.2. Functional Genomic Screening

Beyond simple knockout screens, functional genomics has been extended considerably by newer CRISPR-based perturbation modalities. Base-editor screens, in which a barcoded library of base-editing guide RNAs installs defined point mutations across many genes in parallel, allow researchers to interrogate the functional consequences of specific amino acid substitutions genome-wide, rather than simply asking whether a gene is required at all.<sup>[42]</sup> Single-cell CRISPR screens, which couple genetic perturbation with single-cell transcriptomic or epigenomic readouts, have similarly been used to profile the genetic determinants of chromatin accessibility and gene regulatory networks at unprecedented resolution.<sup>[43]</sup>

These approaches have been applied directly to drug-relevant biology: CRISPR screens performed in three-dimensional cancer spheroid models identified vulnerabilities specific to 3D growth conditions that were not apparent in standard two-dimensional culture, better reflecting the tumor microenvironment relevant to *in vivo* drug response.<sup>[40]</sup> Similarly, genome-wide CRISPR screens in BRAF-inhibitor-resistant melanoma cells identified essential mediators of resistance, providing candidate combination-therapy targets to overcome or delay clinical drug resistance.<sup>[41]</sup> This class of "perturbomics" approach systematically linking genetic perturbation to phenotype is increasingly central to identifying therapeutic targets for cancer, cardiovascular disease, and neurodegeneration.<sup>[8]</sup>

### 3.3. CRISPR-Based Drug Screening

CRISPR screening has also been used to interrogate mechanisms of resistance to existing and investigational drugs directly in a therapeutic context. For example, a genome-wide CRISPR screen was used to identify resistance mechanisms to CD3-bispecific antibody immunotherapy, revealing genes whose loss allowed tumor cells to evade T-cell engaging therapy and informing strategies to overcome this resistance in the clinic.<sup>[44]</sup> More broadly, CRISPR-based selection platforms have been adapted to identify targets of drugs with non-antiproliferative mechanisms of action, extending the applicability of functional genomic screening beyond simple cell-survival phenotypes to a much wider range of therapeutic modalities.<sup>[9]</sup>

### 3.4. Disease Modeling for Drug Discovery

CRISPR-Cas9 has substantially improved the fidelity of disease models used in drug discovery by enabling precise introduction or correction of patient-derived mutations in isogenic cellular backgrounds, removing the confounding genetic variability inherent to comparisons between unrelated patient- and control-derived cell lines.<sup>[1,10]</sup> This capability has been applied across a wide range of disease areas, from monogenic disorders to complex polygenic conditions, and has been particularly valuable in generating human induced pluripotent stem cell (iPSC)-derived disease models that recapitulate specific pathogenic mutations for downstream drug screening.<sup>[1]</sup>

### 3.5. CRISPR-Engineered Cell Models

Beyond disease modeling per se, CRISPR-engineered cell lines are now used routinely to build reporter systems, knock-in fluorescent tags, and generate humanized cell models for pharmacological and toxicological screening throughout the drug development pipeline.<sup>[1,10]</sup> These engineered models allow more predictive, mechanistically grounded evaluation of candidate compounds earlier in development, reducing reliance on animal models and improving the translational relevance of preclinical data before a compound advance to clinical testing.<sup>[1]</sup>

**Table 2: Applications of CRISPR technology in pharmaceutical drug discovery.**

| Application           | CRISPR approach           | Purpose                        | Example                   | Pharmaceutical relevance |
|-----------------------|---------------------------|--------------------------------|---------------------------|--------------------------|
| Target identification | Genome-wide CRISPR screen | Identify essential genes       | Cancer targets            | Target discovery         |
| Target validation     | KO/CRISPRi/CRISPRa        | Confirm target function        | Disease models            | Target validation        |
| Drug resistance       | CRISPR screening          | Identify resistance genes      | BRAF inhibitor resistance | Combination therapy      |
| Drug mechanism        | CRISPR perturbation       | Identify drug targets/pathways | Small molecules           | MoA studies              |
| Disease modelling     | Knock-in/knockout         | Reproduce disease mutations    | iPSC models               | Preclinical testing      |
| Drug screening        | CRISPR-engineered models  | Test candidate drugs           | Organoids                 | Lead evaluation          |

## 4. Therapeutic Applications of CRISPR-Cas Technology

### 4.1. CRISPR-Based Cancer Therapeutics

Cancer has been among the most active therapeutic areas for CRISPR application, particularly through the engineering of chimeric antigen receptor (CAR) T-cell therapies. CRISPR-Cas9 has been used to knock out inhibitory immune checkpoint genes such as *PD-1* and *CTLA-4* in CAR T cells, extending their persistence and antitumor activity in preclinical and early clinical studies.<sup>[45,46]</sup> CRISPR has also enabled the manufacture of "universal" allogeneic CAR T-cell products by disrupting endogenous T-cell receptor and HLA genes to reduce the risk of graft-versus-host disease and immune rejection, addressing a major scalability limitation of autologous, patient-specific CAR T-cell manufacturing.<sup>[47]</sup>

Clinically, an early-phase trial evaluating CRISPR-Cas9 edited autologous T cells (with knockout of the endogenous T-cell receptor, *B2M*, and *PD-1*) in patients with refractory cancer demonstrated feasibility and an acceptable safety profile, with engineered T cells persisting for several months post-infusion.<sup>[49]</sup> Similarly, CRISPR/Cas9-mediated *PD-1* editing of autologous T cells in patients with refractory non-small-cell lung cancer showed low off-target editing rates and no severe treatment-related adverse events in a first-in-human phase I trial.<sup>[50]</sup> CRISPR-based screening has

additionally identified genetic modifications, such as disruption of *RASA2* or *TET2*, that enhance CAR T-cell persistence and antitumor efficacy, informing next-generation cell-engineering strategies.<sup>[48]</sup>

#### 4.2. Treatment of Genetic and Rare Diseases

CRISPR-Cas9 has demonstrated clinical proof-of-concept for a range of monogenic and rare diseases beyond oncology. NTLA-2002, an in vivo CRISPR-Cas9 therapy targeting the *KLKB1* gene in hepatocytes, produced dose-dependent, durable reductions in plasma kallikrein levels and a substantial reduction in attack frequency in a phase 1 trial in patients with hereditary angioedema, illustrating the potential for a single-dose curative approach to a lifelong genetic disease.<sup>[13]</sup> More broadly, the growing pipeline of CRISPR-based therapies for rare monogenic disorders reflects the technology's particular suitability for diseases with a well-defined, single-gene genetic cause.<sup>[11]</sup>

#### 4.3. Hematological Disorders

Hematological disorders represent the most clinically mature application of CRISPR therapeutics to date. Casgevy (exagamglogene autotemcel), the first CRISPR-Cas9 based therapy to receive regulatory approval, edits the *BCL11A* erythroid-specific enhancer in autologous hematopoietic stem cells to reactivate fetal hemoglobin expression, compensating for defective adult hemoglobin in patients with sickle cell disease and transfusion-dependent  $\beta$ -thalassemia; the pivotal trial demonstrated durable clinical benefit and has since been approved in the UK, US, EU, and several other jurisdictions.<sup>[14,51]</sup> A related but mechanistically distinct approach directly disrupts the *HBG1/HBG2*  $\gamma$ -globin gene promoters, identified through a tiling CRISPR-Cas9 screen, to induce fetal hemoglobin expression; this strategy has shown sustained fetal hemoglobin induction and clinical improvement in patients with severe sickle cell disease.<sup>[52]</sup> More recently, a CRISPR-Cas12a edited cell therapy (renizgamglogene autogedtemcel) targeting the same *HBG1/HBG2* promoter region achieved normalization of total hemoglobin and elimination of vaso-occlusive events in the large majority of treated patients, while a base-editing approach targeting the same regulatory region has also entered late-stage clinical evaluation, together illustrating the diversity of editing strategies now converging on this single therapeutic node.<sup>[33,37]</sup>

#### 4.4. Infectious Diseases

CRISPR-based strategies for infectious disease span both direct antiviral gene editing and diagnostic applications. EBT-101, an AAV-delivered CRISPR-Cas9 therapy using dual guide RNAs to excise a large segment of integrated HIV proviral DNA, has been developed as a potential functional-cure strategy for HIV infection and has received FDA Fast Track designation.<sup>[14]</sup> CRISPR-Cas3, a type I CRISPR system with intrinsic long-range DNA degradation activity, has also been explored as a sequence-specific antimicrobial, including phage-delivered CRISPR-Cas3 systems designed to selectively kill *Clostridium difficile* without disrupting the broader gut microbiome.<sup>[6]</sup> In parallel, CRISPR-Cas12 and Cas13 collateral-cleavage activity underlies rapid, high-sensitivity molecular diagnostic platforms (SHERLOCK and DETECTR) that have been applied to pathogen detection, discussed further in Section 7.<sup>[34,35]</sup>

#### 4.5. Metabolic and Neurological Disorders

In metabolic disease, in vivo CRISPR base editing of *PCSK9* produced durable reductions in low-density lipoprotein cholesterol in non-human primates, establishing proof-of-concept for single-dose base-editing therapy for cardiovascular risk reduction.<sup>[36]</sup> Building on this, a first-in-human phase 1 trial of CTX310, a CRISPR-Cas9 therapy targeting hepatic *ANGPTL3*, achieved dose-dependent reductions in ANGPTL3 protein levels alongside clinically meaningful reductions in LDL cholesterol and triglycerides, with no dose-limiting toxicities reported.<sup>[54]</sup> In

neurological disease, CRISPR interference targeting mutant huntingtin (*mHTT*) expression reduced *mHTT* levels in the striatum of Huntington's disease mouse models with fewer off-target effects than conventional Cas9-mediated cleavage, illustrating the potential of reversible, non-cleaving CRISPRi approaches in postmitotic neural tissue where permanent DNA modification carries a less favorable risk-benefit profile <sup>[21]</sup>. CRISPR-Cas9 correction of familial Alzheimer's disease-associated *PSEN2* mutations has similarly been shown to normalize amyloid- $\beta$  processing and associated electrophysiological deficits in patient-derived neurons.<sup>[6]</sup>

#### 4.6. Other Emerging Therapeutic Applications

Beyond these established areas, CRISPR-based approaches are being explored for autoimmune and ophthalmic disease, including EDIT-101, a CRISPR-Cas9 therapy delivered directly to the retina to correct the *CEP290* mutation underlying Leber congenital amaurosis type 10 <sup>[11]</sup>. The breadth of these emerging applications spanning ocular, autoimmune, and other monogenic disease areas reflects the platform's flexibility once a validated genetic target and a suitable delivery route have been established.<sup>[11,16]</sup>

**Table 3: Representative clinical applications of CRISPR-based therapeutics.**

| Disease               | CRISPR approach       | Target     | Delivery    | Development status | Key outcome                    |
|-----------------------|-----------------------|------------|-------------|--------------------|--------------------------------|
| Sickle cell disease   | Cas9                  | BCL11A/HBG | Ex vivo HSC | Approved           | HbF restoration                |
| $\beta$ -thalassemia  | Cas9                  | BCL11A     | Ex vivo HSC | Approved           | Reduced transfusion dependence |
| ATTR                  | Cas9                  | TTR        | LNP         | Clinical           | TTR reduction                  |
| Hereditary angioedema | Cas9                  | KLKB1      | LNP         | Clinical           | Reduced kallikrein             |
| Dyslipidemia          | Cas9                  | ANGPTL3    | LNP         | Clinical           | Lipid reduction                |
| Cancer                | CRISPR-edited T cells | Various    | Ex vivo     | Clinical           | Cell therapy                   |

### 5. CRISPR-Based Gene and Cell Therapies

#### 5.1. Ex Vivo Genome Editing

Ex vivo genome editing involves removing cells from the patient (or a donor), editing them outside the body, and reinfusing the modified cells the strategy underlying Casgevy and CRISPR-engineered CAR T-cell therapies.<sup>[14,49]</sup> This approach offers substantial safety and quality-control advantages, since editing efficiency and specificity can be verified prior to reinfusion, but it is inherently more resource- and time-intensive than in vivo administration, particularly for autologous (patient-specific) products that require individualized manufacturing.<sup>[16,47]</sup> Early-phase trials of CRISPR-edited autologous T cells have demonstrated that ex vivo-edited cellular products can persist for months following infusion with a generally favorable safety profile, though genomic safety monitoring remains essential: a study assessing chromosomal integrity following CRISPR-Cas9 cleavage in primary human T cells found that a subset of cells can acquire whole-chromosome loss (aneuploidy) after editing, underscoring the importance of rigorous genomic quality control in ex vivo-edited cell products intended for clinical use.<sup>[55]</sup>

#### 5.2. In Vivo Genome Editing

In vivo genome editing delivers CRISPR components directly to the patient, most commonly via lipid nanoparticle or AAV-mediated delivery to the liver, avoiding the need for cell harvest and ex vivo manipulation altogether <sup>[23,24]</sup>. This approach has produced some of the most clinically advanced CRISPR therapeutics to date, including nexiguran

zinc finger nucleases for transthyretin amyloidosis, NTLA-2002 for hereditary angioedema, and CTX310 for ANGPTL3-mediated dyslipidemia, all of which use LNP-encapsulated Cas9 mRNA and guide RNA to achieve permanent, single-dose gene disruption in hepatocytes<sup>[12,13,54]</sup>. An earlier proof-of-concept trial of in vivo CRISPR-Cas9 editing for transthyretin amyloidosis similarly demonstrated substantial, dose-dependent reductions in serum transthyretin following a single intravenous infusion, establishing the feasibility of this delivery strategy several years before nexiguran zinc finger nucleases' more extensive phase 1 dataset<sup>[53]</sup>. The consistent hepatic tropism of current LNP formulations means that, to date, in vivo CRISPR therapeutics have been concentrated in diseases where the liver is the relevant target organ; extending in vivo editing to other tissues remains an active area of delivery-technology development, discussed in Section 6.<sup>[23,24]</sup>

### 5.3. CRISPR-Engineered Cell Therapies

Beyond CAR T cells, CRISPR is being used to engineer a broader range of cellular therapeutics, including natural killer (NK) cells and T-cell receptor (TCR)-engineered T cells, with CRISPR-mediated gene knockout and knock-in used to enhance target recognition, persistence, and resistance to tumor-mediated immunosuppression.<sup>[56]</sup> Site-specific CRISPR-mediated integration of CAR constructs into defined genomic loci, such as the *PD-1* or *TRAC* locus, has been shown to produce more uniform, durable CAR expression than conventional random-integration viral delivery, while simultaneously disrupting an endogenous gene relevant to T-cell exhaustion or alloreactivity.<sup>[47,57]</sup> These engineering strategies illustrate how CRISPR's precision allows multiple therapeutic objectives transgene insertion, endogenous gene disruption, and control of integration site to be achieved within a single editing step.<sup>[57]</sup>

### 5.4. Clinical Translation of CRISPR Therapeutics

The clinical translation of CRISPR-based gene and cell therapies has accelerated markedly since Casgevy's approval, with a growing number of in vivo and ex vivo CRISPR therapeutics now in mid- to late-stage clinical trials across hematology, cardiometabolic disease, and rare genetic disorders.<sup>[11,14]</sup> This clinical maturation has been accompanied by growing industry-academia collaboration and an increasingly defined regulatory pathway for genome-editing therapeutics, though significant translational challenges around long-term safety monitoring, delivery specificity, manufacturing scalability, and cost remain and are addressed in Sections 6, 8, and 9.<sup>[11,28]</sup>

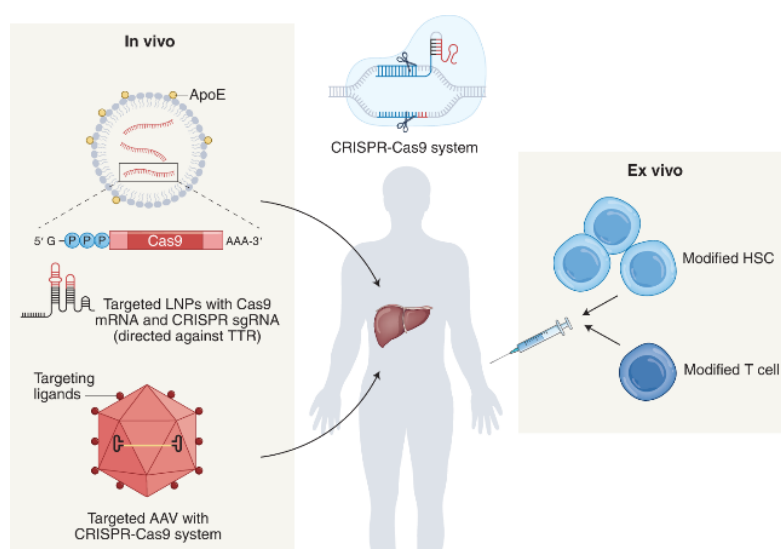


**Figure 4: In Vivo and Ex Vivo CRISPR-Based Genome Editing Strategies.**

## 6. CRISPR Delivery Technologies for Pharmaceutical Applications

### 6.1. Viral Delivery Systems

Adeno-associated virus (AAV) vectors remain the most clinically established viral delivery platform for in vivo CRISPR therapeutics, owing to their broad, serotype-tunable tissue tropism, low pathogenicity, and long track record in approved gene therapies.<sup>[23,29]</sup> However, SpCas9's coding sequence, combined with a single-guide RNA expression cassette, approaches or exceeds AAV's ~4.7-kb packaging limit, forcing trade-offs such as split-intein Cas9 reconstitution, dual-vector delivery, or the use of smaller Cas orthologs (e.g., SaCas9, Cas12a) to fit within a single capsid.<sup>[23]</sup> Large-scale AAV manufacturing also remains a persistent bottleneck: current production methods transient transfection, baculovirus/insect-cell, and stable producer-cell systems each face trade-offs in yield, capsid empty/full ratios, and cost of goods that constrain scalability as clinical demand grows.<sup>[29]</sup> Pre-existing anti-AAV neutralizing antibodies in a substantial fraction of the population further limit redosing and patient eligibility for AAV-delivered editors.<sup>[23,29]</sup>

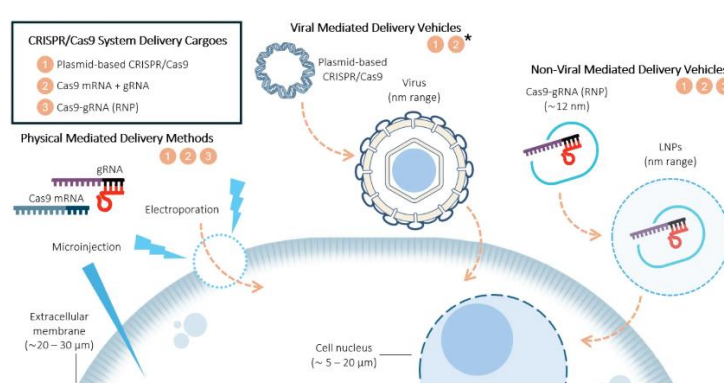


**Figure 5: Delivery Strategies for CRISPR-Cas9 Genome Editing Systems.**

### 6.2. Non-Viral Delivery Systems

Non-viral delivery methods avoid several of the manufacturing, immunogenicity, and cargo-size constraints associated with viral vectors. Electroporation of Cas9 ribonucleoprotein (RNP) complexes pre-assembled Cas9 protein bound to its guide RNA is now the standard method for ex vivo editing of hematopoietic stem cells and T cells, offering rapid intracellular delivery, transient editor exposure that reduces off-target accumulation, and compatibility with GMP-scale, closed-system manufacturing.<sup>[16,23]</sup> For applications requiring in vivo, non-electroporation delivery, polymer- and peptide-based nanoparticles, cell-penetrating peptides, and engineered virus-like particles (VLPs) that package Cas9 RNP or mRNA without viral genetic material are under active development, aiming to combine the transient, low-immunogenicity profile of RNP delivery with the systemic reach of a viral vector.<sup>[23]</sup>

### 6.3. Lipid Nanoparticle-Based Delivery

Ionizable lipid nanoparticles (LNPs) have emerged as the dominant in vivo delivery platform for CRISPR therapeutics, co-encapsulating Cas9 (or base-editor) mRNA with a chemically modified single-guide RNA to achieve transient editor expression confined largely to the liver following intravenous administration.<sup>[24]</sup> This approach underlies nexiguran ziclumeran, NTLA-2002, and CTX310, described in Sections 4 and 5, and offers key advantages over viral delivery: mRNA-based LNP cargo avoids the packaging-size constraints of AAV, editor expression is self-limiting since mRNA degrades within days, and manufacturing does not require cell-based viral production.<sup>[12,13,24,54]</sup> The principal limitation of current ionizable-lipid chemistries is their strong hepatic tropism, driven largely by apolipoprotein E-mediated

uptake in hepatocytes, which has concentrated clinically advanced in vivo CRISPR therapeutics in liver-expressed targets and left extrahepatic tissues comparatively underserved.<sup>[24]</sup>

#### 6.4. Emerging Delivery Strategies

A new generation of delivery strategies aims to extend CRISPR editing beyond the liver and beyond simple gene disruption. Ligand-conjugated and tissue-selective LNP formulations, engineered VLPs capable of packaging full Cas9-RNP or prime-editor complexes, and self-limiting mRNA-encoded delivery of large, multi-component editors such as prime editors and site-specific integrases are collectively working to decouple delivery reach from cargo size and hepatic tropism.<sup>[23,24]</sup> These advances are discussed further, alongside the specific emerging editing platforms that depend on them, in Section 10.4.

**Table 4: Comparison of CRISPR delivery systems.**

| Delivery              | Application     | Advantages              | Limitations            |
|-----------------------|-----------------|-------------------------|------------------------|
| AAV                   | In vivo         | Tissue tropism          | Cargo size, immunity   |
| LNP                   | In vivo         | mRNA delivery, scalable | Liver tropism          |
| Electroporation       | Ex vivo         | Efficient RNP delivery  | Mainly ex vivo         |
| Polymer nanoparticles | In vivo/ex vivo | Flexible                | Translation challenges |
| VLPs                  | Emerging        | Transient delivery      | Development stage      |

### 7. CRISPR-Based Diagnostics and Precision Medicine

#### 7.1. CRISPR-Based Molecular Diagnostics

Beyond genome editing, the collateral (non-specific) nuclease activity triggered by CRISPR-Cas12 and Cas13 upon recognition of a complementary target sequence has been harnessed to build rapid, highly sensitive nucleic acid diagnostic platforms. SHERLOCK (Cas13-based) and DETECTR (Cas12-based) couple isothermal amplification with CRISPR-guided target recognition to generate a fluorescent or lateral-flow readout, enabling pathogen detection with sensitivity approaching quantitative PCR but without the need for thermal cycling equipment, making these platforms well suited to point-of-care and resource-limited settings.<sup>[34,35]</sup> These diagnostic platforms have been validated for detection of SARS-CoV-2, influenza, and other viral pathogens, and are increasingly being adapted into multiplexed panels capable of distinguishing related pathogens or viral variants within a single assay.<sup>[34]</sup>

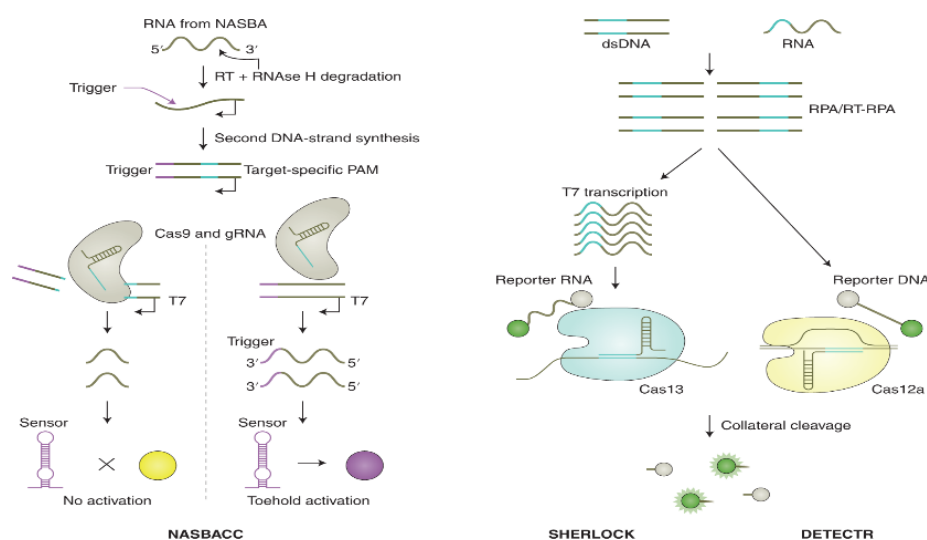


**Figure 6: CRISPR-Based Molecular Diagnostic Platforms: NASBA-CRISPR, SHERLOCK, and DETECTR.**

## 7.2. CRISPR in Biomarker Discovery

Genome-wide and targeted CRISPR screens, described in Section 3 in the context of drug target identification, are equally applicable to biomarker discovery: by systematically perturbing genes and correlating the resulting phenotype with a molecular or clinical readout, CRISPR screening can identify genetic determinants of drug sensitivity or resistance that serve as predictive or pharmacodynamic biomarkers for patient stratification.<sup>[7,8]</sup> Single-cell CRISPR screens extend this further by linking a given genetic perturbation directly to its transcriptomic or epigenomic consequences, allowing biomarker candidates to be nominated alongside a mechanistic rationale for their association with treatment response.<sup>[43]</sup>

## 7.3. CRISPR in Personalized Medicine

CRISPR technology intersects with personalized medicine both through patient-specific ex vivo cell therapies, in which a patient's own cells are edited and returned as an autologous product tailored to that individual, and through pharmacogenomic applications that use CRISPR screening to map how specific genetic variants influence drug metabolism and response.<sup>[10]</sup> Artificial-intelligence-assisted analysis of pharmacogenomic and CRISPR screening data is increasingly used to prioritize which genetic variants are functionally relevant to drug response, helping translate large-scale genomic datasets into actionable, patient-specific treatment guidance.<sup>[10]</sup>

## 7.4. Integration of CRISPR with Precision Therapeutics

CRISPR-based diagnostics and CRISPR-based therapeutics are beginning to converge into integrated precision-medicine workflows, in which rapid molecular diagnostics confirm a genetic or infectious diagnosis, and in some cases quantify a relevant biomarker, before or during administration of a matched CRISPR-based or conventional therapy.<sup>[11,34]</sup> This convergence is most advanced in infectious disease, where CRISPR-based diagnostics can rapidly confirm the causative pathogen ahead of a targeted antimicrobial or antiviral CRISPR intervention (Section 4.4), and is expected to extend further as companion-diagnostic-style genetic testing becomes standard practice ahead of gene-editing therapies for hematological and metabolic disease.<sup>[11]</sup>

# 8. Challenges in the Clinical and Pharmaceutical Translation of CRISPR

## 8.1. Off-Target and Unintended Genetic Effects

Off-target editing is the modification of genomic sequences that closely, but imperfectly, resemble the intended target site remains the most extensively studied safety concern for CRISPR-based therapeutics.<sup>[25,26]</sup> Because even low-frequency off-target edits in a therapeutic cell or tissue population could, in principle, disrupt a tumor-suppressor gene or activate an oncogene, regulatory agencies expect comprehensive off-target characterization before clinical use. A range of unbiased, genome-wide detection methods including GUIDE-seq, CIRCLE-seq, and Digenome-seq have been developed to empirically map candidate off-target sites beyond what computational prediction alone can identify, and are now considered standard components of the preclinical safety package for CRISPR therapeutics.<sup>[25,27]</sup> Continued refinement of high-fidelity Cas9 variants and improved guide RNA design algorithms (Section 10.2) has substantially reduced, though not eliminated, off-target editing frequency in current clinical candidates.<sup>[27]</sup>

## 8.2. Delivery and Tissue-Specificity Challenges

As detailed in Section 6, achieving efficient, tissue-specific delivery while minimizing off-target tissue exposure remains one of the most significant unsolved challenges in CRISPR therapeutic development.<sup>[23,24]</sup> Current in vivo delivery technologies are effectively confined to the liver (via LNP or AAV) or to cells that can be removed, edited ex

vivo, and reinfused; extending efficient, safe delivery to other clinically important tissues including the central nervous system, skeletal muscle, and solid tumors will require delivery vehicles capable of crossing additional biological barriers, such as the blood-brain barrier, without triggering excessive innate immune activation.<sup>[23,24,29]</sup>

### 8.3. Immunogenicity and Safety Concerns

Immune responses represent a further layer of safety risk distinct from off-target genetic effects. Cas proteins are of bacterial origin and can be recognized as foreign by the adaptive immune system; pre-existing humoral and T-cell immunity to Cas9, arising from prior bacterial exposure, has been documented in a meaningful proportion of the general population and could in principle blunt efficacy or trigger adverse immune reactions upon in vivo administration.<sup>[16]</sup> Viral capsid proteins in AAV delivery, and to a lesser extent lipid nanoparticle components, can independently provoke innate immune activation and complement-mediated infusion reactions, requiring careful immunogenicity monitoring and, in some cases, prophylactic immunosuppression during dosing.<sup>[16,23]</sup>

### 8.4. Genome Stability and Long-Term Effects

Double-strand break-dependent editing carries a risk of unintended large-scale genomic consequences beyond simple off-target mutation, including large deletions, chromosomal translocations, and chromothripsis at the cut site.<sup>[15,25]</sup> Of particular relevance to ex vivo cell therapies, a study directly assessing chromosomal integrity in primary human T cells following CRISPR-Cas9 cleavage found that a measurable subset of edited cells acquired whole-chromosome loss (aneuploidy), underscoring the need for rigorous genomic quality-control assays beyond conventional off-target sequencing before edited cell products are released for clinical use.<sup>[55]</sup> Activation of the p53-mediated DNA damage response following double-strand breaks has also been shown to select against efficiently edited cells with intact p53 function, raising a theoretical concern that surviving edited cells could be enriched for reduced p53 activity, an issue that is comparatively less pronounced with nick-based editors such as base and prime editing.<sup>[15,55]</sup>

### 8.5. Manufacturing and Scalability Challenges

Many of the most clinically mature CRISPR therapeutics rely on autologous, patient-specific manufacturing: cells are harvested from an individual patient, edited, expanded, quality-tested, and reinfused into that same patient, a process that typically takes weeks, requires dedicated cleanroom capacity per patient batch, and carries substantial per-patient cost.<sup>[16,28]</sup> Allogeneic, "off-the-shelf" cell products manufactured at scale from a single donor source offer a potential solution but require additional editing steps to prevent graft-versus-host disease and immune rejection, adding manufacturing and regulatory complexity of their own.<sup>[47]</sup> In vivo therapies avoid patient-specific cell manufacturing but shift the scalability burden onto viral vector or LNP production, both of which face their own capacity, cost-of-goods, and batch-consistency constraints as clinical programs move toward larger patient populations.<sup>[28,29]</sup>

**Table 5: Major challenges in pharmaceutical translation of CRISPR and potential strategies.**

| Challenge          | Problem                        | Potential strategy                          |
|--------------------|--------------------------------|---------------------------------------------|
| Off-target editing | Unintended mutations           | High-fidelity Cas, improved gRNA design     |
| Delivery           | Poor tissue specificity        | Targeted LNPs/VLPs                          |
| Immunogenicity     | Immune response                | Alternative Cas proteins/transient delivery |
| Genome instability | Large deletions/rearrangements | Base/prime editing                          |
| Manufacturing      | High cost/complexity           | Automation, allogeneic approaches           |
| Long-term safety   | Unknown delayed effects        | Extended genomic monitoring                 |
| Cost/access        | Very expensive therapies       | Scalable manufacturing/payment models       |

## **9. Ethical and Regulatory Perspectives**

### **9.1. Ethical Considerations in Genome Editing**

The clinical advance of CRISPR-based therapeutics has been accompanied by sustained ethical scrutiny, particularly concerning informed consent for irreversible, single-administration genetic interventions, equitable access to extremely high-cost therapies, and the long-term societal implications of normalizing genome editing as a treatment modality.<sup>[30,31]</sup> A systematic review of the ethical literature on human genome editing highlights persistent disagreement over where the boundary between therapeutic correction of disease-causing mutations and non-therapeutic genetic enhancement should be drawn, and over how much weight should be given to the interests of future generations who cannot themselves consent to heritable changes.<sup>[30]</sup>

### **9.2. Somatic versus Germline Editing**

A foundational ethical and regulatory distinction separates somatic editing, which alters only the treated individual's own cells and is not transmitted to offspring, from germline editing, which alters the genome of embryos, gametes, or early-stage reproductive cells in a manner that is heritable.<sup>[30]</sup> Every CRISPR therapeutic discussed elsewhere in this review from Casgevy to the in vivo hepatic editing programs is a somatic intervention, and this distinction underlies why somatic CRISPR therapeutics have progressed to regulatory approval while human germline editing remains prohibited or under moratorium in the overwhelming majority of jurisdictions, following widespread condemnation of the 2018 report of germline-edited infants in China.<sup>[30]</sup>

### **9.3. Regulatory Challenges in CRISPR-Based Therapies**

Regulatory frameworks for gene-editing therapeutics have evolved considerably since Casgevy's approval, with agencies such as the FDA and EMA developing genome-editing-specific guidance covering preclinical off-target characterization, long-term follow-up requirements, and manufacturing consistency standards, but substantial heterogeneity remains across jurisdictions in how CRISPR-edited products are classified and reviewed.<sup>[32]</sup> Because most approved and late-stage CRISPR therapeutics are directed at rare or ultra-rare diseases, regulators have increasingly relied on accelerated approval pathways and surrogate or intermediate clinical endpoints, a practice that accelerates patient access but places additional weight on robust post-marketing surveillance to confirm durable clinical benefit and long-term safety.<sup>[32]</sup>

### **9.4. Clinical Safety and Risk Assessment**

Ongoing clinical experience across the expanding portfolio of CRISPR therapeutics is progressively strengthening the field's risk-assessment framework. Longer-term follow-up of nexiguran ziclumeran, including its extension into patients with hereditary transthyretin amyloidosis complicated by polyneuropathy, has continued to demonstrate durable, dose-dependent reductions in serum transthyretin without new safety signals emerging with extended exposure and an expanded indication, reinforcing confidence in the single-dose in vivo editing paradigm as clinical experience accumulates.<sup>[61]</sup> Task forces convened to examine the affordability and post-marketing infrastructure surrounding CRISPR genomic therapies have similarly emphasized the need for coordinated, long-term registries capable of detecting rare or delayed adverse events across an increasingly diverse set of approved and investigational gene-editing products.<sup>[28]</sup>

## 10. Emerging Trends and Future Perspectives

### 10.1. Base and Prime Editing

Base and prime editing continue to expand the precision genome-editing toolkit beyond nuclease-mediated cleavage. Since the original description of prime editing, which uses a Cas9 nickase fused to a reverse transcriptase and a prime-editing guide RNA (pegRNA) to directly install a specified edit without a double-strand break or donor DNA template, engineering efforts have focused on improving editing efficiency and delivery compatibility.<sup>[15,20]</sup> Structurally engineered pegRNAs with optimized secondary structure and stability have been shown to substantially increase prime editing efficiency across diverse genomic targets, while delivery of prime editors as pre-assembled ribonucleoprotein complexes rather than as plasmid or viral constructs has demonstrated efficient, transient editing in zebrafish and primary human cells with reduced off-target activity, paralleling the RNP-delivery advantages already established for standard Cas9 editing.<sup>[58,59]</sup> In parallel, CRISPR-directed integrase systems capable of inserting large DNA sequences at a targeted genomic locus without generating a double-strand break represent a further extension of nick-based editing principles to applications such as gene addition and correction of large structural variants that remain beyond the practical reach of base or prime editing alone.<sup>[18,19,60]</sup>

### 10.2. AI-Assisted CRISPR Design

Machine learning models are increasingly integrated into guide RNA design and off-target risk prediction, moving beyond simple sequence-similarity heuristics to learn complex, data-driven relationships between guide RNA sequence, chromatin context, and empirically observed on- and off-target activity.<sup>[38,39]</sup> Explainable deep-learning architectures, which provide interpretable rationale alongside their predictions rather than functioning as opaque black boxes, are being developed specifically to support the regulatory-grade off-target risk assessments required for clinical CRISPR programs, addressing a key barrier to fully automating guide RNA selection for therapeutic use.<sup>[38]</sup> These AI-assisted design tools are also being extended to pharmacogenomic applications, helping prioritize which genetic variants identified through CRISPR screening are most likely to be functionally consequential for drug response.<sup>[10]</sup>

### 10.3. Precision and Personalized Genome Editing

CRISPR interference and activation (CRISPRi/CRISPRa) technologies, which use catalytically dead Cas9 to modulate gene expression reversibly without altering the underlying DNA sequence, are positioned to play a growing role in precision and personalized editing strategies where a permanent, irreversible genetic change is undesirable.<sup>[21,22]</sup> Because CRISPRi/a effects are tunable and reversible, these approaches are particularly well suited to postmitotic tissues, such as the central nervous system, and to disease contexts in which dosage correction, rather than permanent gene disruption or correction, is the therapeutic goal.<sup>[21]</sup> Combined with genetic and pharmacogenomic patient stratification, these reversible modulation strategies point toward a future in which the choice of editing modality is cleavage-based, base-editing, prime-editing, or CRISPRi/a is itself individualized to a patient's specific mutation and clinical context.<sup>[10,21]</sup>

### 10.4. Advanced CRISPR Delivery Platforms

Building on the delivery technologies described in Section 6, next-generation platforms are focused on extending CRISPR delivery beyond the liver and beyond simple nuclease cargo. Engineered virus-like particles and tissue-selective lipid nanoparticle chemistries aim to achieve extrahepatic and cell-type-specific targeting, while the compact, non-viral RNP and mRNA delivery formats now validated for prime editors and CRISPR-directed integrases suggest

that increasingly sophisticated, multi-component editing machinery can be delivered without reliance on viral vectors or their associated packaging and immunogenicity constraints.<sup>[23,24,58,60]</sup> Collectively, these advances are expected to progressively decouple the reach of CRISPR delivery from both target tissue and editor complexity.

#### **10.5. CRISPR in Next-Generation Drug Discovery**

CRISPR-based functional genomics is expected to remain a core pillar of pharmaceutical target discovery, with continued growth in single-cell, spatial, and in vivo CRISPR screening approaches that generate progressively richer, more physiologically relevant functional genomic datasets.<sup>[7,8,9]</sup> Integration of these functional genomic datasets with AI-assisted analysis is beginning to support more systematic prioritization of novel drug targets and prediction of on-target efficacy and off-target liability earlier in the discovery pipeline, potentially compressing the time and cost required to advance a genetically validated target into a clinical candidate.<sup>[9,10]</sup>

#### **10.6. Prospects for Clinical Translation**

The trajectory from Casgevy's approval through the growing portfolio of in vivo hepatic editing programs suggests that CRISPR-based therapeutics are transitioning from a novel treatment modality to an established, if still specialized, pillar of pharmaceutical development.<sup>[11,14]</sup> Continued clinical maturation will likely depend on resolving the manufacturing, delivery, and affordability challenges detailed in Sections 6, 8, and 9, alongside the continued expansion of harmonized regulatory pathways capable of accommodating the growing diversity of editing modalities are nuclease, base-editing, prime-editing, and reversible CRISPRi/a-based now advancing toward the clinic.<sup>[11,28,32]</sup>

#### **11. Limitations of This Review**

Several limitations should be considered when interpreting the scope and conclusions of this review. First, CRISPR-Cas genome editing is an unusually fast-moving field: new editing modalities, clinical trial results, and regulatory decisions continue to be published on a near-monthly basis, and any narrative synthesis is therefore a snapshot that will require periodic updating rather than a static, permanent account of the field's state.<sup>[11,14]</sup> Second, this review draws predominantly on English-language, peer-reviewed literature and publicly available clinical trial reporting; results disclosed only in conference abstracts, regulatory filings, or non-English-language sources, as well as unpublished negative or null results, are underrepresented, which may bias the review toward positive findings and clinically successful programs a form of publication bias inherent to narrative reviews of clinical technologies.

Third, the breadth of this review, spanning mechanism, drug discovery, therapeutics, delivery, diagnostics, ethics, and regulation, was prioritized over exhaustive depth in any single subtopic; readers seeking a comprehensive treatment of a specific area, such as AAV capsid engineering or the regulatory history of a single approved therapy, are encouraged to consult the specialized literature cited throughout. Fourth, because clinically advanced CRISPR therapeutics are concentrated in a small number of well-resourced companies and a limited set of hepatic and hematological targets, conclusions drawn about the field's translational trajectory may not generalize equally well to less-resourced indications, geographies, or delivery targets where development has been slower. Finally, this review does not include a formal, systematic literature search with defined inclusion and exclusion criteria; sources were selected for relevance and recency rather than through a systematic or meta-analytic methodology, and the review should therefore be read as a narrative synthesis rather than a systematic review.

## CONCLUSION

CRISPR-Cas genome editing has progressed, within little more than a decade, from a bacterial adaptive immune mechanism to a validated pharmaceutical platform spanning drug discovery, direct genetic therapeutics, and molecular diagnostics. Across the sections of this review, three themes recur. First, the continued diversification of the CRISPR toolbox from nuclease-based cleavage to base editing, prime editing, CRISPRi/a, and site-specific integrases is steadily expanding the range of genetic changes that can be made safely and precisely.<sup>[2,15,18,60]</sup> Second, delivery technology, rather than the editor itself, increasingly represents the rate-limiting step in translating a validated editing strategy into a deliverable therapeutic, particularly for indications beyond the liver.<sup>[23,24]</sup> Third, the field's clinical maturation marked by Casgevy's approval and the expanding pipeline of in vivo hepatic editing programs has been accompanied by an evolving, but still incomplete, framework for addressing the safety, manufacturing, ethical, and affordability challenges that will determine how broadly these technologies benefit patients.<sup>[11,16,28,32]</sup> Realizing CRISPR's full pharmaceutical potential will depend on continued progress across all of these dimensions in parallel, rather than on further advances in editing precision alone.

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