

CRISPR-Based Epigenome Editing for Precision Medicine: Molecular Mechanisms, Therapeutic Applications, Delivery Challenges, and Future Clinical Translation

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Abstract

Cancer, neurologic diseases, cardiovascular disease, metabolic disorders, immune disorders, and some inherited diseases are examples of epigenetic diseases. Traditional epigenetic drugs are non-specific and may be toxic at higher concentrations, may be unstable in transcription and may have off-target effects in normal tissues. An epigenome editor consists of an epigenome modifier (e.g., DNA methylation or demethylation, and histone-modification domain or transcription regulation domain) fused to a catalytically inactive Cas protein, typically nuclease-dead Cas9 (dCas9), which is a more precise method. With these systems, certain genes can be turned on or off and controlled by a programmable single-guide RNA, which does not cause permanent double-strand breaks in DNA. Locus-specific and, in some instances, heritable gene expression regulation has been shown by various experimental platforms such as dCas9-DNMT3A, dCas9-TET1, dCas9-p300, dCas9-LSD1, dCas9-KRAB, and CRISPRoff/ CRISPRon. Potential applications for the preclinical use of these include reactivation of tumour suppressor genes, silencing of oncogenic pathways, regulation of FMR1 expression in fragile X syndrome, interference with fibrotic and inflammatory pathways, and the generation of stable immune-cell phenotypes. The drawbacks of these epigenetic-editor constructs include delivering them to tissues, resistance to the Cas proteins, possible off-target chromatin changes, inconsistent duration of effect, and the lack of regulatory parameters. This review focuses on the molecular basis, therapeutic applications, delivery systems, safety and translational needs of epigenome editing with CRISPR. When combined with the power of AI, single-cell multi-omics, small Cas proteins, cell-inducible editors and biomarker-guided patient selection, epigenome editing may emerge as a programmable element of precision medicine—potentially reversible.

Keywords: CRISPR/dCas9; epigenome editing; DNA methylation; histone modification; precision medicine; gene regulation

1. Introduction

Epigenetic regulation refers to heritable or persistent alterations of the activity of genes that do not involve a change in the nucleotide sequence. The combination of DNA methylation, histone modification, nucleosome

positioning, chromatin remodelling and higher-order genome organisation is responsible for the activation, poising or repression of a region of the genome. Epigenetic changes can block tumour suppressor genes, activate oncogenic programmes, alter expression of genes in neurons, alter the expression of genes in immune cells and alter their responses to environmental and therapeutic factors. The epigenome may be an important source of disease biomarkers and therapeutic targets because of these features. An epigenetic change differs from a pathogenic change of DNA, which in theory could be reversible, with the restoration of physiological expression of a gene, without permanently altering it [1,2].

Epigenetic drugs that have been used traditionally are DNA methyltransferase inhibitors, histone deacetylase inhibitors, histone methyltransferase inhibitors, and bromodomain inhibitors. Numerous are clinically active, particularly in hematological malignancies. These drugs, however, have many genomic targets and can be cytotoxic, modulate the expression of genes that are not related, have a short duration of action, and can be tissue-specific. To resolve this limitation, Precision epigenome editing was developed, which targets a specific regulatory activity to a selected regulatory element, such as a promoter, enhancer, imprinting-control region, and so on [2,3].

The initial use of the CRISPR-Cas system was to create a genome editing tool that can cleave sequence-specific DNA [4]. To create catalytically dead dCas9, mutations were then introduced into the RuvC and HNH nuclease domains of Cas9. dCas9 is unable to cut genomic DNA but is able to bind to a genomic site controlled by a single-guide RNA. As such it can be used as a programmable carrier for transcriptional repressors, activators, as well as DNA methyltransferases, DNA demethylases, histone-modifying enzymes and chromatin-remodelling factors [5,6]. In contrast to zinc-finger proteins and transcription activator-like effectors [1,3], the targeting sequence can be modified by redesigning the guide, bringing a practical advantage to CRISPR.

CRISPR interference and CRISPR activation, which do not change the DNA sequence of the endogenous gene, were first shown to inhibit and activate endogenous genes, respectively, with the same ability to inhibit and activate foreign genes [5,6]. Epigenetic editors went one step further and deposited or removed particular chromatin marks. Examples of these include dCas9-DNMT3A for DNA methylation, dCas9-TET1 for DNA demethylation, dCas9-p300 for histone acetylation, dCas9-LSD1 for histone demethylation and dCas9-KRAB for recruitment of repressive complexes [7-13]. Newer multivalent platforms have the ability to create a memory of the transcription that lasts beyond the editor's lifespan [14,15].

This is an important distinction between genome and epigenome editing, as it can lead to clinical effects. When a mutation in a pathogen needs to be corrected, genome editing may be used to produce a permanent change in the sequence. Epigenome editing can be employed to alter gene regulation; this can be beneficial in diseases where a problem is abnormal gene expression, dosage imbalance, an enhancer defect, imprinting or reversible chromatin state (see Table). It can also permit a step-down process, not a knockout. Some, however, are reversibly altered to a stable mitotic state; others are not. Thus, an epigenome editing approach based on CRISPR technology should not be taken for granted as being safe or completely reversible [14-16].

Proof of concept and therapeutic safety of the molecule is highly required. The majority of studies have been conducted in cultured cells, organoids or small animal models. In humans, the functional properties of the antibody, tissue specificity and immunogenicity remain somewhat mysterious. This review covers the mechanisms underlying CRISPR epigenome editing, the potential applications in precision medicine, delivery and safety issues, and what scientific research needs to do to facilitate the translation of CRISPR epigenome editing to the clinic.

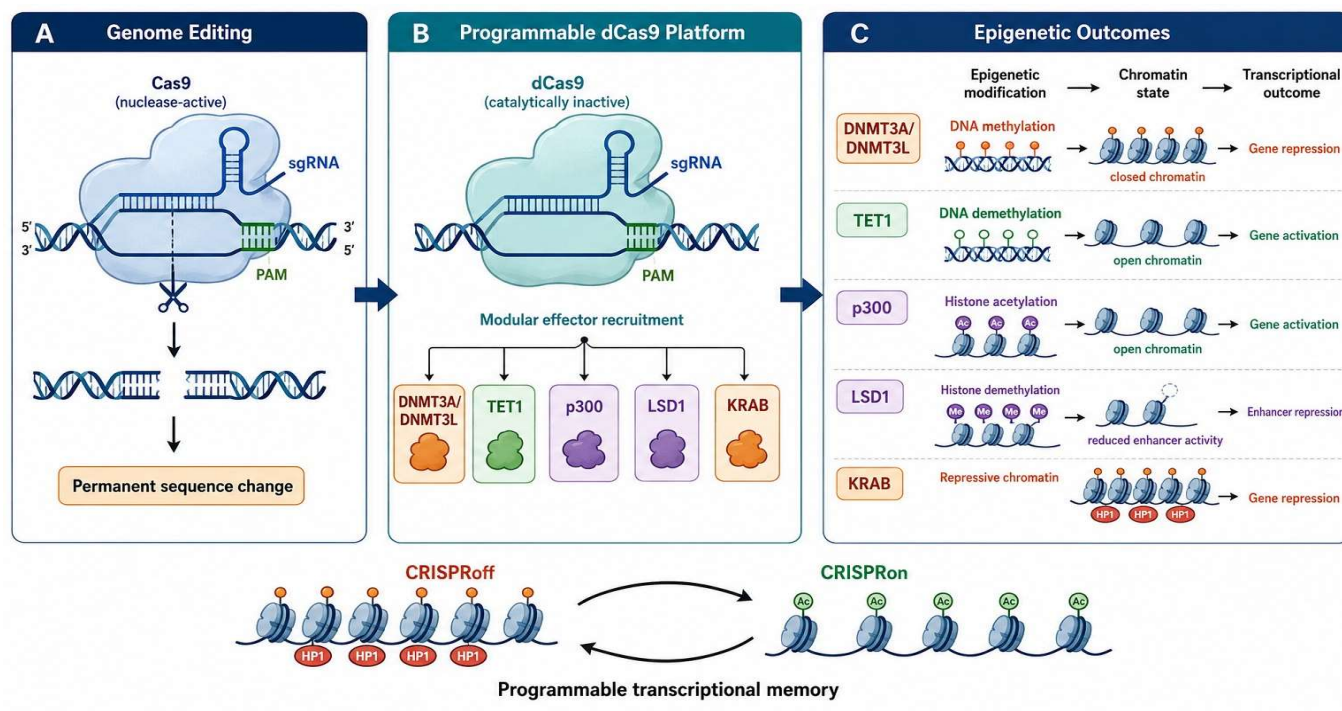


Figure 1. Evolution and molecular architecture of CRISPR-based epigenome editing. The proposed figure should illustrate nuclease-active Cas9, dCas9-directed recruitment of DNMT3A/DNMT3L, TET1, p300, LSD1 and KRAB, and the transcriptional outcomes of CRISPRoff/CRISPRon.

2. Molecular Mechanisms of CRISPR-Based Epigenome Editing

2.1 CRISPR/dCas9 targeting platform

The conventional CRISPR-Cas9 complex consists of sgRNA, which is made up of targeting and scaffold regions, and Cas9. sgRNA finds complementary DNA within about 20 nucleotides of a protospacer-adjacent motif. While dCas9 binds without breaks, nuclease-active Cas9 makes double-strand breaks. This programmable binding allows steric interference of transcription or delivery of an effector function to a regulatory element [4-6]. Thus, modulation of gene expression can take place along different molecular pathways by targeting promoters, enhancers, silencers, insulators or transcription-factor-binding sites.

The dCas9-KRAB is a protein that is frequently used for CRISPR interference. The Krüppel-associated box brings corepressors and helps to establish repressive chromatin, such as H3K9 trimethylation. The system can also impact chromatin accessibility and transcription when targeted to transcription start sites or regulatory enhancers. CRISPR activation systems use VP64, p65, RTA or synergistic activation mediator complexes, such as VPR. These systems involve the importation of endogenous transcription machinery, but do not necessarily generate a specific epigenetic mark [6,17]. Epigenome editors are designed to extend this activity by binding to DNA and/or histones and adding or removing defined DNA/epigenetic modifications, which could persist after the editor has been removed.

2.2 Targeted DNA methylation

DNA methylation at CpG sites in the promoter region of genes is often associated with transcriptional repression, but the role of methylation varies with the chromosomal environment. The catalytic domain of DNMT3A can be

fused with dCas9 to enable the targeting of specific loci to a methylation readout. Vojta et al. showed endogenous promoter methylation, and Stepper et al. optimised the efficiency by using a DNMT3A-DNMT3L setup [9,12]. DNMT3L alone is catalytically inactive, but it is able to boost the activity of DNMT3A and either expand or stabilise targeted methylation.

It is possible that specific methylation can be used to suppress disease-associated genes without inducing any breaks in the DNA. However, silence after deposition is not a sure thing. The chromatin accessibility, transcription factor occupancy, CpG density, cell division and maintenance (DNMT1) affect persistence. The methylation may be extended beyond the methylation region, and there may be no apparent link between the specific methylation and transcription. A fusion protein consisting of dCas9 and MQ1, a bacterial methyltransferase, was developed by Lei et al., and they showed in vivo targeted methylation, emphasising the importance of activity testing outside the guide binding site [16].

Amabile et al. were able to demonstrate that exposing the cells to the editor and two chromatin regulators, the DNA methylation and repressive chromatin regulators, was enough for inheritable silencing [10]. CRISPRoff wrapped up the KRAB, DNMT3A and DNMT3L activities and designed programmable transcriptional memory (cell division and differentiation) to many human genes. A matching CRISPRon strategy led to the reversal of selected silenced states [15]. The systems of this kind are attractive for long-term treatment, but have safety issues related to the adverse effects following the removal of the editors.

2.3 Targeted DNA demethylation

TET proteins catalyse active DNA demethylation, which is the oxidation of 5-methylcytosine. The fusion of the catalytic domain of TET with dCas9 enables the locus-specific demethylation and possible reactivation of epigenetically silenced genes. Liu et al. edited the DNA methylation landscape in mammalian cells, and Xu et al. showed targeted DNA demethylation with dCas9-TET1 [8,11]. Choudhury et al. proposed a model by which they activated the expression of the tumour suppressor gene BRCA1 through selective demethylation and enhanced expression of the gene by targeting TET1 to the promoter of the BRCA1 gene [18].

The question of whether methylation of promoters occurs in a causal fashion to cause repression or it is merely a result of repression has important ramifications for the biological outcome. When silencing is achieved by histone modifications, nucleosome structure or transcriptional repressors, demethylation might be inadequate. Some platforms are designed to couple TET1 with transcriptional activators or to employ several guides within a regulatory area, all of which accomplish the same objective. Multiplex targeting can be more effective but also makes the construct more complex and there are more potential off-target sites.

2.4 Histone acetylation, deacetylation and methylation

Overall, acetylation of histones is correlated with active chromatin and transcription. To activate expression of endogenous genes using their promoters and from distant enhancers, Hilton et al. fused the catalytic domain of p300 to dCas9 [7]. The system controlled local acetylation of the protein H3K27 and was used as a tool to give a causal function to non-coding regulatory elements. It can also reactivate the expression of beneficial genes that are normally quiescent as a result of abnormal gene silencing during chromatin compaction.

This can also be accomplished for histone demethylases. To remove the enhancer-associated methylation and to find functional enhancers and control expression levels of the target genes, Kearns et al. designed a fusion protein between dCas9 and LSD1 [13]. Depending on the residue that is methylated, specific histone methylation may either turn genes on or turn them off. Cano-Rodriguez et al. showed that the trimethylation of H3K4 occurs during the deposition and that extended expression of this mark was still restricted by the chromatin context [19]. O'Geen

et al. also reported that the acquisition of repressive histone methylation is not always enough in order to achieve stable repression [14]. The therapeutic editors are linked in a network, and epigenetic marks may need to be coordinated in the network with the recruitment of transcription factors.

2.5 Chromatin organisation and transcriptional memory

Three-dimensional relationships between promoters, enhancers, silencers and insulators also regulate gene expression. Such dCas9 systems can be employed to target architectural proteins or to modify methylation at CTCF binding sites that can alter chromatin looping. This is a way to regulate genes that are targeted by pathogenic enhancer contacts, but it can also be a way to regulate many genes in the same chromosomal domain. In order to be used in therapeutics, the chromatin conformation and transcriptome analyses are necessary.

One of the great objectives is the principle of transcription memory. A short exposure that produces a stable therapeutic level may minimise repeated dosage and cumulative toxicity. The long-term effects are not known and temporary may be safer. The timing and extent of editing will be controlled by "inducible, light-responsive, drug-controlled and self-limiting platforms" that are being developed [2,15]. Table 1. Major CRISPR-based epigenome editors, mechanisms, therapeutic opportunities and limitations.

Editor/platform	Principal action	Expected effect	Potential application	Major limitation
dCas9-DNMT3A/DNMT3L	Deposits CpG methylation	Gene repression	Silencing oncogenes or pathogenic alleles	Variable durability and methylation spreading
dCas9-TET1	Promotes targeted demethylation	Gene reactivation	Restoring tumour-suppressor or imprinted-gene expression	May require additional chromatin remodelling
dCas9-p300	Deposits activating histone acetylation	Gene activation	Activating therapeutic genes and enhancers	Possible activation of nearby elements
dCas9-LSD1	Removes enhancer-associated methylation	Enhancer repression	Functional enhancer targeting	Strong chromatin-context dependence
dCas9-KRAB	Recruits repressive complexes	Transcriptional repression	Silencing disease-associated genes	Effects may spread locally
CRISPRoff/CRISPRon	Writes or reverses epigenetic memory	Stable repression or reactivation	Long-term programmable control	Persistence complicates reversal

3. Therapeutic Applications in Precision Medicine

3.1 Cancer epigenetics and precision oncology

Cancer is a result of a combination of genetic and epigenetic abnormalities. Promoter hypermethylation may lead to the repression of tumour-suppressing genes, and enhancer reprogramming and abnormal histone modification may lead to the activation of oncogenic networks. Systemic epigenetic drugs are not locus-specific and may be able to reverse some changes. Epigenome editing with CRISPR, on the other hand, might be used to re-activate a specific tumour suppressor, suppress a dominant oncogene, or block a cancer-specific enhancer, while sparing effects on healthy cells [3,21].

In cancer models, targeted TET1-mediated demethylation has been able to activate BRCA1 and other silenced genes [18]. dCas9-p300 can induce the activation of genes through acetylation of their promoters or enhancers, while the dCas9-DNMT3A and KRAB systems can prevent the activation of oncogenes. Such an approach is interesting because malignant phenotypes can usually be maintained by a transcriptional programme and not just one druggable mutation. There is a challenge of tumour heterogeneity – not all clones will be targeted by the same target; editing pressure may favour resistant populations.

The molecular selection in precision oncology will be based on causal epigenetic lesions. In addition, whole genome bisulphite sequencing, chromatin-accessibility profiling, histone-mark mapping, spatial transcriptomics and single-cell sequencing can differentiate driver abnormalities from passenger changes. Patient-derived organoids can then be used to determine if editing a candidate state results in a sustained anti-tumour response. The combinations with immunotherapy, chemotherapy, targeted therapy or cellular therapy might be more effective than epigenome editing alone [21,22].

3.2 Neurological and neurodevelopmental disorders

Neurological diseases represent promising targets which, however, are technically more challenging due to the fact that neuronal identity is based on stable, cell-specific epigenetic programmes. In fragile X syndrome, the increase in CGG repeats results in promoter hypermethylation and transcription silencing of the FMR1 gene. Liu et al. showed that demethylation of the FMR1 gene by dCas9-TET1 improved electrophysiological abnormalities in differentiated patient-derived neurons and re-expressed FMR1 in patient-derived cells [23]. This study revealed that it is possible to reverse a disease-relevant epigenetic state by editing the disease without removing the repeat expansion.

Other indications include MECP2 disease, Huntington's disease, Alzheimer's disease, Parkinson's disease and neuropsychiatric diseases. An inhibitory editor could dissipate a neurotoxic dominant gene, while a promoting editor can enhance a missing gene which is essential for protection. Neurons are non-dividing and long-lived; this makes unintended changes potentially lasting for years. One major challenge is getting the drug to the brain through the blood–brain barrier. Direct intracranial administration is invasive, and neurotropic viral vectors could lead to long-term expression and immune risk. Investigations into the use of nanoparticles and engineered EVs for systemic or intrathecal delivery are underway [24,25].

3.3 Cardiovascular and metabolic diseases

Cardiovascular and metabolic diseases are the result of interaction between epigenetic regulation, ageing, diet and inflammation, as well as inherited susceptibility. Possible targets are suppression of proatherogenic genes, modulation of lipid metabolism, reversal of maladaptive cardiac remodeling, and regulation of fibrosis. For targeted hydroxymethylation, Xu et al. found that it is possible to restore gene expression and reduce renal fibrosis in vivo by targeting fibrosis-specific epigenetic changes [26]. These approaches might also be relevant to cardiac and vascular fibrosis.

The effects of the activation of metabolic pathways that are beneficial toward health, or the repression of a pathogenic regulatory element, might lead to durable regulation without the necessary modification of coding sequences. Most cardiovascular and metabolic diseases, however, are polygenic and affect multiple tissues. Compared to other cells, lipid nanoparticles are more easily delivered to hepatocytes, but are less easily delivered to cardiomyocytes, endothelium, adipose tissue and pancreatic cells. Evidence that the epigenetic abnormality targeted is causal, modifiable, and sufficiently important to modify clinical outcomes is needed for patient selection.

3.4 Inherited and rare disorders

Epigenome editing is applicable to diseases associated with gene-dosage imbalance, imprinting defects, haploinsufficiency or allele-specific silencing. An editor might not modify all of the pathogenic mutations, but rather enhance expression from a functional gene and might act to silence a dominant toxic allele while leaving the normal allele untouched. Guides that recognize a pathogenic variant or associated polymorphism can be used to achieve allele specificity; however, genetic diversity may necessitate reagents that are patient-specific.

Imprinting disorders are potential targets because disease could occur due to aberrant methylation at an imprinting-control region. Multiple genes within the same domain could be influenced by disruption and may be restored to physiological dosage by editing such regions. There are specific molecular targets for rare diseases, and they are challenging to recruit, produce and evaluate over time. A platform where only the guide is modified – and the editor is fixed – may have a shorter development time, but all guides would still need to be assessed for potency, specificity and toxicology.

3.5 Immune, inflammatory and infectious diseases focus on diseases that involve the immune system, inflammation and infection.

Epithelial programmes are stable but adaptable, controlling immune-cell states. These programmes may be edited to help to boost antitumoral immunity, dampen pathological inflammation, or stabilise therapeutic phenotypes. To strengthen Foxp3 expression in primary T cells, Okada et al. applied dCas9 epigenome editing to Foxp3 [28]. Adoptive cellular therapies might be prevented from becoming exhausted, persist better, or have less expression of inhibitory receptors by related strategies.

The ex vivo approach is particularly promising, as immune cells or haematopoietic stem cells can be isolated, manipulated in the lab under controlled conditions, examined for their potency and for potential off-target effects, and re-infused. There must be no unintended changes in the risk of differentiation or transformation. Epigenetic repression could also inhibit host factors that are needed for viral infection or could silence integrated viral genomes. This is limited by viral evolution and the physiological requirements of the host targets; however, multiplex targeting can make viral escape more difficult but lead to increased specificity and production complexity.

4. Delivery Strategies, Safety Challenges and Ethical Considerations

4.1 Viral delivery systems

To carry the dCas9, effector domains and sgRNAs to the target cell. Adeno-associated virus (AAV) is widely utilised because of its tissue tropism and gene-therapy experience, and its relatively small size is an obstacle to larger dCas9-effector fusions. This can be overcome by dual-vector systems and split-intron plus compact cas orthologues, but will compromise the efficiencies due to the fact that both halves need to be delivered to the same cell [24,29]. Lentiviral vectors for ex vivo engineering provide greater capacity and long-term expression, but have problems with insertional and long-term expression. Adenoviral vectors are larger, but may cause more immune response.

4.2 Non-viral delivery systems and nanocarriers.

Lipid nanoparticles, polymeric nanoparticles, inorganic particles, cell-penetrating peptides and extracellular vesicles are examples of non-viral platforms. They are able to deliver plasmid DNA, messenger RNA, or guide RNA. Temporary editor expression is achieved via messenger RNA, and no vector integration, which could help minimise long-term off-target effects. The long transcript of the dCas9 is unstable and immunostimulatory, however [25,29,30]. At present, efficient delivery to the liver is accomplished by lipid nanoparticles; the lipid composition, surface ligands, and properties may be used to expand the targeting capability to other organs. Specificity can be further enhanced with tissue-specific promoters, receptor-targeting ligands and local administration.

4.3 Off-target epigenetic activity

Evaluation of off-target effects should not be limited to off-target Cas binding. A single effector can modify the chromatin structure around low-affinity binding sites and can impact neighboring genes. In addition, dCas9 editors do not need breaks, but can identify nuclease off-target activity [31,32]. Whole genome methylation profiling, chromatin immunoprecipitation sequencing, ATAC sequencing, RNA sequencing, and, if applicable, chromosome conformation analysis should be included in the assessment.

Carefully designed guides, truncated guides, high fidelity/Cas or compact constructs, paired targeting and transient delivery are all methods to enhance specificity. Multiple independent guides should yield the same phenotype and should separate the effects of epigenetic activity from steric interference: the former should be blocked by catalytically inactive effector controls. The durability of altered expression after editor withdrawal and the changes over time need to be evaluated in long-term studies.

4.4 Immunogenicity and biological safety

Cas proteins are derived from bacteria, and pre-existing adaptive immunity against the most commonly used orthologues of Cas9 has been found in humans [33]. Edited cells can be killed by the immune system, or their efficacy can be lowered, and inflammation or inability to be given again may result. These can be attempted by using less commonly used or engineered Cas proteins, transient expression, immune shielding, tissue-restricted delivery, and ex vivo editing. Another factor of safety is based on the target biology: excess activation of a growth-promoting gene may lead to malignancy, while excess repression of an essential gene may lead to unhealthy tissue function. Genotoxicity, tumorigenicity, cytokine responses, cell identity and reproductive toxicity should be considered when applicable and evaluated in studies.

4.5 Ethical and regulatory considerations

Ethically, there is a difference between somatic epigenome editing for disease treatment and heritable germline epigenome editing. Given their potential to pass down unpredictable effects across generations, embryo and germline interventions must not be carried out without broad societal consensus and tight governance. Somatic applications continue to raise questions regarding long-term monitoring and informed consent with regard to unknown future risks, access and management of persistent epigenetic changes. Standards will need to be developed for epigenome editing that will be required by regulators. While traditional gene-therapy approaches focus on issues of vector biodistribution, genotoxicity and immune responses, there are additional endpoints that need to be considered for locus-specific methylation, histone modification, chromatin structure, transcriptional memory and reversibility. To ensure reproducibility and public trust, all guide sequences, delivery systems, target engagement data and genome-wide safety data need to be reported transparently.

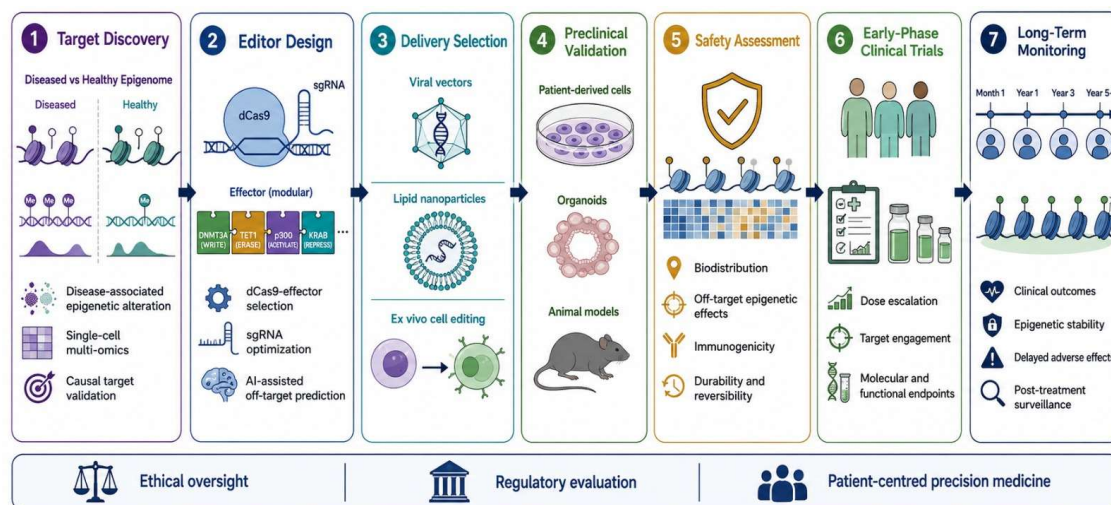


Figure 2. Translational framework for CRISPR-based epigenome editing: biomarker discovery, editor design, delivery selection, patient-derived model testing, animal studies, genome-wide safety evaluation, early-phase trials and long-term monitoring.

5. Future Clinical Translation and Conclusion

The next generation of editors should be more powerful and compact, have limited activity and be easily reversible. The ability to provide several effector molecules in a single construct may be made possible by the help of the Compact Cas proteins, while engineered recruitment systems can be used to provide multiple effector molecules without undue increase in the size of the construct. Activity-limiting inducible editors can only be activated within a therapeutic window, depending on the drug, light, or temperature, or the disease. In addition to these brief exposures, there are also constructs that self-destruct and RNA (messenger) systems that are transient, which could be utilised.

Incorporating the principle of sequence complementarity, chromatin accessibility, DNA methylation, nucleosome positioning, tissue-specific transcription, and predicted off-target activity could also be used to guide AI-based selection of guides. The multi-omics models could be useful to distinguish 'causal epigenetic drivers' from the 'epigenetic marks' associated with disease but not functional. Despite their predictive power, computational predictions should still be validated experimentally, as the chromatin states vary between different cell types, between individuals and between the different stages of a disease.

Clinical translation will likely begin when it is easiest to deliver and monitor, that is, ex-vivo edited blood / immune cells or locally available tissues / liver-directed therapies. Early trials should be targeted to serious diseases on which there is good mechanistic evidence, limited treatment and measurable molecular endpoints. A staged programme should be shown to achieve delivery, engagement of the target, transcription, functional benefit and clinical improvement. If stable epigenetic memory or integrating vectors are used, long-term follow-up will be important.

If patient stratification is done on the basis of these biomarkers, this technology will become precise. Patients should be selected according to a validated pathogenic epigenetic state, and not a diagnosis. Locus-specific assays, transcript and protein measurements, functional biomarkers and conventional clinical outcomes should be used for treatment response monitoring. Individualised evidence would be obtainable before treatment using patient-derived organoids and induced pluripotent stem-cell models.

In summary, dCas9 can be used as a programmable epigenome editing tool to write, erase or read regulatory information in the absence of DNA sequence editing. The selected genes can be switched on, off or recalled by dCas9-DNMT3A, dCas9-TET1, dCas9-p300, dCas9-LSD1, dCas9-KRAB and CRISPRoff/CRISPRon. The preclinical results have resulted in applications in oncology, neurological disease, fibrosis, inherited disorders, and immune-cell engineering. The targeted cell delivery, detailed off-target analyses, extended timeline, immunological safety, and causality of the targeted alteration as a disease-causing factor will affect therapeutic efficacy. Epigenome editing has the potential to be a clinically relevant sub-branch of precision medicine, enabled by compact and non-viral approaches, single-cell multi-omics, artificial intelligence, and stringent regulatory requirements.

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