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Precision Cell Fate Engineering with CRISPR Epigenetic Editing for Regeneration and Off-the-Shelf Immunotherapy

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Abstract. Induced pluripotent stem cell (iPSC)-based regenerative medicine and cellular immunotherapy are severely hindered by heterogeneous cell phenotypes, unstable cellular functions and inconsistent large-scale manufacturing. Different from traditional CRISPR/Cas9 genome editing that induces DNA double-strand breaks and genomic risks, dCas9-mediated epigenetic editing offers a way to modulate gene regulation through targeted changes in DNA methylation and chromatin state without intentionally cutting the DNA. This review systematically summarizes core research progress and key findings in this field: targeted epigenetic modification mediated by CRISPRoff can establish persistent transcriptional memory during iPSC differentiation, supporting more stable control of cell state; IL1RN-targeted methylation editing of the promoter directly links epigenetic regulation to altered myeloid cell immune functions, providing evidence for a causal link between epigenetic state and cellular phenotype. The RENDER virus-like particle delivery platform may help address the cell-viability limitations associated with electroporation, realizing efficient and low-toxicity epigenetic editing in primary T cells. Combined with iPSC master cell bank technology, epigenetic editing may support more standardized expansion and batch-based production of CAR-T/CAR-NK off-the-shelf products. This review further clarifies existing limitations including off-target epigenetic effects, residual undifferentiated iPSCs and immune rejection, and proposes a systematic development strategy integrating precise epigenetic regulation, safe delivery and standardized iPSC manufacturing, providing a potential pathway for the clinical translation of cell fate engineering.

Keywords: iPSC, CRISPR epigenome editing, cell fate engineering, tissue regeneration, off-the-shelf immunotherapy

1. Introduction

With the rapid development of cell-based therapeutics, regenerative medicine and tumor immunotherapy have become increasingly important therapeutic approaches alongside small-molecule treatments, whose therapeutic efficacy is fundamentally determined by cellular identity, gene expression profile and phenotypic stability [1]. As ideal seed cells for clinical cell preparation, iPSCs have substantial self-renewal capacity and multi-directional differentiation potential, enabling early genetic and epigenetic modification at the pluripotent stage to avoid the heterogeneity of

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primary cells derived from individual donors. Nevertheless, conventional differentiation strategies relying on growth factor induction may not consistently generate homogeneous, fully mature cell populations, while autologous immune cell therapy suffers from long preparation cycles and poor batch consistency, which can complicate large-scale clinical translation. Standardized, universal and safe engineered cell products have therefore become an important clinical goal in the cell therapy industry [1].

CRISPR epigenetic editing, represented by dCas9, has expanded the capabilities of CRISPR beyond some of the limitations of traditional DNA-cleavage editing [2]. Different from Cas9-mediated permanent gene knockout, nuclease-dead dCas9 targets specific gene loci via guide RNA and regulates transcription activity by modifying DNA methylation and chromatin conformation without introducing an intentional DNA double-strand break. Recent studies have confirmed the durability and heritability of epigenetic editing: the CRISPRoff system can establish stable transcriptional memory during iPSC neuronal differentiation, and targeted methylation modification can be stably inherited in human hematopoietic and immune cell lineages [3, 4]. Functionally, epigenetic editing of the locus has been reported to regulate myeloid-cell inflammatory responses, confirming the functional causality of epigenetic regulation [5]. In terms of delivery technology, the RENDER platform achieves high-efficiency transient editing while maintaining high cell viability, potentially reducing the viability loss associated with traditional electroporation [6]. However, current studies often focus on individual technical components, while the integration of epigenetic editing with iPSC manufacturing remains less explored.

Despite the above technical advances, a critical gap remains between precise molecular epigenetic editing and safe, scalable clinical cell therapy. Many studies do not yet fully address the safety risks of persistent off-target epigenetic effects and residual undifferentiated iPSCs, and standardized manufacturing systems remain under development. To address these deficiencies, this review systematically constructs a complete research framework centered on "precision epigenetic regulation and standardized iPSC manufacturing". Firstly, it distinguishes the mechanistic differences between genome editing and epigenetic editing; secondly, it demonstrates the causal relationship between epigenetic modification, gene expression and cell functional phenotype; finally, it analyzes the application advantages and safety challenges of integrating dual technologies in tissue regeneration and off-the-shelf immunotherapy, and proposes optimized translational strategies for clinical industrialization.

2. From DNA cleavage to programmable epigenetic regulation

The main difference between genome editing and epigenome editing is what is being changed. With conventional Cas9, a DNA double-strand break is deliberately introduced and the cell's repair machinery determines the final sequence outcome. That strategy is powerful when a permanent mutation or gene knockout is required, but the break itself introduces an additional potential source of genomic instability. dCas9 takes a different route. It can be guided to a selected locus without cutting the DNA, and an attached effector can then change transcription or the local epigenetic environment [2, 6, 7].

Cai et al. describe several versions of this idea, including CRISPR interference, CRISPR activation, DNA methylation editing, and histone modification. In these systems, dCas9 is better understood as a targeting scaffold than as a nuclease [2]. Fadul et al. place the same tools in a broader context, covering their use in disease studies, functional genomics, and regenerative medicine [7]. The important point is that the same targeting principle can be connected to different

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effectors depending on whether the goal is repression, activation, methylation, demethylation, or another form of chromatin regulation.

There is, however, a trade-off that is easy to miss. Avoiding a DNA double-strand break does not mean that the biological effect is necessarily short-lived. The CRISPRoff study showed that a temporary treatment can leave a persistent transcriptional memory [3]. From a therapeutic perspective, this could be useful because a cell may not need to carry an active editor for its entire life. At the same time, a wrongly targeted epigenetic change could remain after the original editing components have disappeared. That makes the control of duration and reversibility an important part of safety assessment.

For cell-fate engineering, the attraction of epigenome editing is therefore broader than simply saying that it does not cut DNA. It provides a way to change the regulatory state of a gene at a chosen location and, depending on the editor, to make that change temporary or relatively persistent. This is relevant to differentiation because cellular identity depends on coordinated gene networks rather than on one isolated sequence. A method that changes those regulatory networks without permanently altering genomic sequence could be useful when the desired outcome is a controlled shift in cell state.

3. Epigenetic editing provides a mechanistic route to cell-fate control

Differentiation is governed by networks of transcription factors, enhancers, promoters, and chromatin states. For this reason, changing the regulatory environment of a gene can sometimes be more useful than simply deleting or inserting a sequence. Epigenome editing fits this logic because it can focus directly on the regulatory layer that determines whether a gene is accessible and expressed. The CRISPRoff work is an important example. Nunez et al. developed a dCas9-based system that could establish DNA methylation together with repressive histone modifications. The resulting repression was not limited to the period when the editor was present. It could be retained during cell division and through differentiation into neurons, showing that a defined epigenetic state can become part of a more stable cellular program [3].

Saunderson et al. extended this idea to human hematopoiesis. Their results indicated that targeted methylation changes could survive differentiation and remain detectable in downstream immune cells [4]. This matters for cell-fate engineering because it suggests that an intervention made in an earlier precursor population can influence the behavior of later cell states. In an iPSC system, that could in principle allow selected regulatory programs to be established before the cells are differentiated into a therapeutic lineage. The study by Valcarcel et al. provides an even clearer connection between an epigenetic modification and cell function. Editing the IL1RN promoter with a dCas9-based system altered local DNA methylation and produced a large increase in IL1RN expression. The study also reported downstream changes in myeloid-cell characteristics and inflammatory behavior [5].

What makes this result useful for the present discussion is the sequence of events: a targeted regulatory change was made first, gene expression changed next, and a functional cell phenotype changed after that. That is the type of evidence needed if epigenome editing is to be considered a cell-engineering strategy rather than simply a molecular assay. This evidence is especially valuable for the present research question because it demonstrates a chain of causality: targeted epigenetic modification can alter gene expression, which can alter cell state, which can alter cellular function. Such a chain provides stronger support for therapeutic cell-fate engineering than merely showing that CRISPR can change methylation levels.

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It is tempting to compare genome editing and epigenome editing by asking which one gives a higher editing percentage. That comparison is too narrow for a therapeutic cell product. The outcome also depends on how many cells survive the procedure, whether the desired state remains stable, whether unwanted loci are affected, and whether the process can be repeated at manufacturing scale. The RENDER study makes this point clear. In reporter and cell-line experiments, engineered particles carrying different epigenome editors produced high levels of target silencing, with several conditions reaching more than 75 percent silencing and some large-scale experiments approaching or exceeding 90% [6]. These results show that transient delivery is not necessarily limited to very small laboratory cultures.

The primary T-cell results were more complicated. Under the conditions tested, electroporation of CRISPRoff mRNA gave stronger short-term silencing for the CD55 and CD81 targets than RENDER, but electroporation also caused much greater loss of cell viability. For the reported comparison, silencing was about 21 percent with RENDER versus about 97 percent with electroporation for CD55, and about 35 percent versus 78 percent for CD81. At the same time, viability loss with high-dose RENDER remained below 5 percent, whereas electroporation caused roughly 40 percent cell death in that experiment [6].

These data do not support the simple claim that one delivery system is universally more efficient. Instead, they show why efficiency has to be defined in a wider way. A method that gives high molecular editing but destroys a large part of the starting population may not produce more usable therapeutic cells. In contrast, a somewhat lower editing level can still be valuable when the cells remain healthy and the effect lasts long enough for the intended application. For this reason, a more practical measure of therapeutic performance would combine target regulation, viable-cell recovery, durability, specificity, and process scalability. This broader definition also makes it easier to compare genome editing, epigenome editing, and different delivery platforms without reducing the discussion to a single percentage.

4. iPSCs convert precision editing into a scalable manufacturing platform

The value of iPSCs becomes clearer when the problem is viewed from a manufacturing perspective. A primary immune-cell product usually begins with material collected from an individual patient. An iPSC platform allows an engineered clone to be expanded before differentiation, which changes the workflow from repeated patient-specific engineering to a bank-based system. Xue et al. describe iPSCs as a source for several engineered immune-cell types, including T cells, NK cells, macrophages, and dendritic cells [8]. The advantage is not only the range of possible products. Because the engineering step can occur at the pluripotent stage, the resulting clone can be selected, expanded, and tested before it is turned into a final therapeutic cell type. That creates a much earlier quality-control checkpoint than a workflow in which every patient's cells are modified separately.

This idea is especially attractive for CAR-NK therapy. Lin et al. discuss iPSC-derived CAR-NK cells as a potentially standardized source of engineered NK cells that can be expanded for repeated use [9]. Compared with a patient-specific product, a banked iPSC source offers a more consistent starting material and could support manufacturing of multiple doses from the same engineered line. NK cells also have biological characteristics that make them interesting for allogeneic use, although questions about persistence, tumor trafficking, and manufacturing remain.

Cichocki et al. emphasize the importance of banking and clonal selection in iPSC-derived NK- and T-cell therapies. The basic manufacturing idea is straightforward: engineer the pluripotent cell, select a well-characterized clone, expand it, and use that master line for repeated differentiation [10]. This does not remove all variation, because differentiation and downstream processing still need

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tight control, but it shifts a significant part of the variability into an upstream stage where it can be tested before large-scale production.

Fang et al. describe a similar concept for allogeneic CAR-cell therapy and discuss iPSCs as a scalable and editable platform. Their review covers strategies such as TRAC disruption, HLA-related immune-evasion approaches, and IL-15 armoring [11]. These modifications show that iPSCs can serve as a central engineering hub where several therapeutic properties are designed before the cells enter the differentiation and manufacturing process.

The significance of this platform is therefore broader than simple cell expansion. iPSCs allow therapeutic engineering to be transferred from a patient-by-patient model to a master-bank model. The tissue-repair and immunotherapy applications are different in their immediate goals, but the engineering problem is similar. In both cases, the final cell needs a defined identity and function, and unwanted cell states need to be minimized. In regeneration, the priority is often lineage purity, maturation, and safe integration with damaged tissue. In immunotherapy, the product must recognize a target, remain functional, and avoid excessive or uncontrolled activity.

Razavi et al. review CRISPR-based tissue-engineering work across a wide range of tissues, including neural, retinal, muscle, bone, cartilage, and skin applications [1]. The review shows that programmable editing can be used at several stages of regenerative workflows, from modifying donor cells to changing pathways involved in tissue repair. It also highlights a recurring issue: generating the correct molecular phenotype is only one part of regeneration. The cells must still mature, interact with their environment, and function as tissue rather than as a collection of differentiated cells.

The same distinction appears in immune-cell engineering. An iPSC-derived CAR-T or CAR-NK product can be designed to recognize a tumor antigen, but antigen recognition alone does not guarantee therapeutic performance. The cell also needs the right level of persistence, cytotoxic activity, and resistance to inhibitory signals. The iPSC platform is useful because several of these properties can be engineered at the source before the final cell is produced [8-11].

Dashti et al. provide a useful comparison from the perspective of CRISPR-engineered mesenchymal stromal/stem cells. Their review discusses approaches intended to reduce immune recognition and improve regenerative or anticancer functions [12]. Importantly, it also shows that immune evasion is not solved by changing one immune-related gene. Cells with modifications involving B2M, CIITA, or related pathways can still interact with the host immune system in complex ways. This is a reminder that a successful cell therapy needs more than a good editing target; it needs a design that works in the biological environment where the cells will actually be used.

CRISPR-engineered mesenchymal stromal/stem cells provides a useful comparison because it shows how different cellular platforms can be engineered to reduce immunogenicity and enhance therapeutic properties. Strategies summarized in that review include modification of major immune-recognition pathways and inflammatory responses. However, the review also highlights an important limitation: immune evasion is not guaranteed simply by deleting one or two immune-recognition components. In reported models, cells carrying immune-evasion modifications and additional engineering strategies could still be rejected, indicating that host-cell interactions are substantially more complex than a single-gene solution [12]. Taken together, these studies suggest that the safest strategy is not to treat cell identity and immune compatibility as the same engineering problem. Epigenetic editing may help define the state and function of a cell, while additional genome engineering or process controls may be needed to manage immune recognition and other product characteristics.

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5. Durability is both the strength and the weakness of epigenetic editing

Durability has both benefits and risks in therapeutic epigenome editing. Persistent epigenetic memory may help maintain an intended cell state during differentiation, which is useful for cell-fate engineering. However, unplanned persistent epigenetic alterations may remain in cell progeny after editing components clearance, creating additional long-term safety risks beyond those associated with transient gene regulation. Such heritable chromatin and methylation modifications across cell differentiation make durability an essential consideration in clinical safety evaluation.

Targeted epigenetic modulation at specific gene loci can effectively reshape cellular gene expression profiles and remodel immune and inflammatory phenotypes, highlighting the regulatory capacity of epigenome editing. The persistent nature of these modifications requires comprehensive safety evaluation beyond routine genomic sequencing. Normal DNA sequence cannot rule out abnormal epigenetic networks and cellular functional disorders, so multi-dimensional detection of methylation status, transcriptomic characteristics and cell activity is important for clinical product validation.

Scalable clinical manufacturing demands balanced performance in editing efficiency, cell viability and batch consistency. Traditional electroporation delivers high short-term editing efficacy yet causes severe cell death, limiting large-scale clinical application. The RENDER transient delivery system may help address this bottleneck by achieving low-toxic multiplexed epigenetic editing in primary immune cells, retaining high cell activity while ensuring effective target regulation, which greatly adapts to standardized industrial manufacturing workflows.

The combination of transient epigenetic editing and standardized iPSC master cell banks could contribute to a more scalable manufacturing workflow for cell therapy. Engineered iPSC clones undergo rigorous pluripotent-stage characterization and stability verification before differentiation, replacing inefficient personalized cell engineering. This bank-based approach may reduce some sources of batch variation and improve the repeatability of engineered cell products, helping move epigenetic editing toward a more integrated manufacturing workflow.

Therapeutic editing performance evaluation should focus on comprehensive product quality rather than single editing efficiency. Qualified clinical cell products require stable phenotypes, high cell viability, durable editing effects and scalable production capacity. Integrating well-characterized iPSC banks, controllable epigenetic regulation, low-toxic delivery and multi-layer quality control supports the shift from simple gene modulation toward more standardized cellular product manufacturing.

6. Optimization strategies and translational outlook

Future research should shift focus from single-locus editing optimization to systematic industrial improvement. Core directions include optimizing delivery systems to balance editing efficacy and cell viability, refining precise epigenetic durability control, and establishing unified manufacturing specifications for iPSC-derived cell products. Continuous optimization of editing cargo and guide design will further expand the clinical applicability of epigenetic editing technology.

Whole-process dynamic quality monitoring is essential to ensure product safety and stability. Heritable epigenetic alterations require long-term detection of target loci and genome-wide epigenetic profiles to avoid unintended regulatory abnormalities. Rigorous pre-production verification of iPSC clonal stability, differentiation potency and immune compatibility eliminates potential clinical risks, while standardized cell bank construction can support more consistent batch quality when appropriate controls are in place.

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In regenerative medicine, epigenetic cell fate control needs further integration with 3D culture and functional maturation systems that more closely reproduce relevant tissue microenvironments. Current epigenetic regeneration protocols lack unified industrial standards. Future clinical translation relies on full-chain optimization of editing tools, delivery strategies, differentiation procedures and quality inspection to achieve stable preparation of high-quality engineered therapeutic cells.

7. Conclusion

This paper systematically clarifies the unique value and translational potential of CRISPR-mediated epigenetic editing combined with iPSC manufacturing technology for precise cell fate engineering in regenerative medicine and off-the-shelf tumor immunotherapy. Different from traditional DNA-breaking genome editing, dCas9-based epigenetic regulation can enable targeted modification of DNA methylation and chromatin state without introducing an intentional DNA double-strand break, which enables stable fixation of cellular phenotypes during iPSC differentiation and establishes clear causal links between epigenetic states and immune cell functions. Advanced transient delivery systems may improve the balance between editing efficiency and cell viability by reducing some of the viability challenges associated with electrical transfection, while standardized iPSC master cell bank construction creates a potential route from patient-specific preparation toward batch-based production and supports the industrialization of universal CAR-T and CAR-NK cell products. This integrated framework provides a promising strategy for overcoming phenotypic heterogeneity and functional instability that restrict clinical cell therapy application. Despite these promising advances, persistent off-target epigenetic modifications, residual undifferentiated iPSCs, and complex host immune rejection still hinder large-scale clinical translation, and current studies lack standardized manufacturing and safety verification systems for epigenetic cell engineering. Future research should focus on optimizing targeted editing specificity, improving multi-dimensional safety monitoring systems, and establishing complete industrial manufacturing specifications to further promote the clinical application and broader clinical use of epigenetic cell fate engineering technology.

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