Engineering CAR-T Cells Based on PRIME Editing Technology: A New Strategy to Overcome T Cell Exhaustion through PD-1 Promoter Modification

Yifan Li

Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
Email: u202415610@hust.edu.cn

***Abstract*—Chimeric Antigen Receptor (CAR)-T cell therapy has achieved remarkable success in the treatment of hematological malignancies, but its efficacy in solid tumors is still severely limited by T cell exhaustion. As one of the core regulators of T cell exhaustion, Programmed Cell Death Protein 1 (PD-1) plays a key role in this process. However, current targeting strategies for PD-1 still have inherent limitations, making it difficult to strike a balance between effective anti-tumor and maintenance of immune homeostasis. This article proposes a new regulatory strategy that uses PRIME editing technology to precisely modify the PD-1 promoter. As a new generation of gene editing tools, PRIME can achieve precise replacement at the single nucleotide level without inducing DNA double-strand breaks. By precisely modifying key regulatory elements in the PD-1 promoter region, this strategy can moderately downregulate PD-1 expression and effectively alleviate T cell exhaustion while retaining partial PD-1 function to maintain immune homeostasis. This regulatory idea provides a new technical path and theoretical support for the future construction of universal CAR-T cells with low exhaustion and high persistence.**

***Keywords*—Chimeric Antigen Receptor (CAR)-T, PRIME, Programmed Cell Death Protein 1 (PD-1) promoter, T cell exhaustion, gene editing**

I. Introduction

Chimeric Antigen Receptor (CAR)-T therapy has achieved remarkable results in hematological malignancies but faces severe limitations in solid tumors due to rapid T cell exhaustion after entering the tumor microenvironment. As a core regulator of T cell exhaustion, Programmed Cell Death Protein 1 (PD-1) is a key therapeutic target for optimizing CAR-T function. T cell exhaustion refers to a state in which T cells gradually lose proliferation and cytotoxicity under chronic antigen stimulation, accompanied by impaired cytokine secretion and shortened survival. PD-1 is continuously overexpressed on exhausted T cells; the binding of its ligands initiates inhibitory signals that suppress T cell activation, proliferation, and cytotoxicity, and PD-1 expression levels are positively correlated with the severity of T cell exhaustion. Most existing PD-1 targeting strategies directly target the PD-1 protein itself but fail to precisely regulate its expression at the transcriptional level. Studies have found that single nucleotide variations in the PD-1 promoter region can modulate its expression, providing a new direction for modest PD-1 regulation through promoter modification [1, 2]. As a next-generation gene editing tool, PRIME editing enables precise single-nucleotide modification without DNA double-strand breaks, offering strong technical support for this regulatory strategy. This study explores a new method to engineer CAR-T cells through PRIME editing-mediated PD-1 promoter modification to moderately regulate PD-1 expression, alleviating exhaustion while retaining partial PD-1 function to maintain immune homeostasis [3, 4].

II. PRIME Editor: A New Generation of T Cell Engineering Tools

A. Principles and Advancement of Gene Editing Technology

Gene editing technology enables precise modification of genome sequences through knockout, insertion, or replacement. From early zinc finger nucleases and TALENs to CRISPR/Cas9, successive iterations have improved editing efficiency and targeting. CRISPR/Cas9 uses gRNA to guide Cas9 nuclease for double-strand cleavage, leveraging endogenous repair for genetic modification, which has greatly lowered technical barriers and enabled widespread T cell engineering. However, DNA double-strand breaks can lead to error-prone DNA repair, off-target effects, and genotoxic risks, thereby hindering safe clinical translation. Against this background, PRIME editing emerged as a next-generation technology that addresses these key limitations while maintaining high targeting and editing efficiency [5].

Manuscript received May 8, 2026; accepted June 8, 2026; published June 19, 2026.

B. How it Works, Advantages, and Limitations

PRIME editing is an optimized CRISPR/Cas9-based technology consisting of a fusion protein of engineered reverse transcriptase and nCas9 nuclease, along with a specialized gRNA. It operates without DNA double-strand breaks: gRNA targets a specific genomic sequence, nCas9 creates a single-strand nick, and reverse transcriptase integrates the target nucleotide sequence into the nick site using the gRNA as a template. Cellular single-strand repair mechanisms then achieve precise genome modifications, including single-nucleotide substitutions and small insertions or deletions. Compared with traditional gene editing, PRIME offers significant advantages: single-strand nicks avoid genotoxicity and off-target mutations; it enables precise single-nucleotide regulation; and it does not require homologous recombination, allowing diverse modification types with broader applicability. However, limitations remain; overall editing efficiency, especially for large fragments, is lower than CRISPR/Cas9; gRNA design and intracellular stability present technical challenges; and the large fusion protein size complicates delivery, particularly in primary immune cells.

C. Current Status of Application in Immune Cells

Leveraging its precision and safety advantages, PRIME editing has been increasingly applied to immune cell engineering in recent years. Research focuses primarily on T cells and Natural Killer (NK) cells for tumor immunotherapy and autoimmune disease treatment. In T cells, PRIME has been used to precisely modify immune checkpoints and antigen receptor genes, enabling quantitative regulation of inhibitory receptor expression to alleviate exhaustion while maintaining immune homeostasis. In NK cells, it enhances tumor-targeted killing by modulating activation and inhibitory receptors. Although PRIME editing in immune cells is still in the early basic and preclinical research stages, recent comprehensive reviews have systematically summarized its potential for engineering next-generation T cell immunotherapies, underscoring its advantages in precision, safety, and versatility [6].

III. PRIME PRINCIPLES AND STRATEGIES FOR EDITING PD-1 PROMOTER

A. Reasons for Targeting Promoters

Precise modification of the PD-1 promoter is a key strategy to overcome the current limitations of PD-1 regulation and achieve quantitative expression control. This approach offers higher precision and controllability compared with direct intervention in the coding region. As the core region of PD-1 transcription, the promoter's sequence characteristics directly determine the transcription efficiency and protein expression level. Studies have confirmed that single-nucleotide variations in this region can significantly alter PD-1 transcriptional activity, providing a theoretical basis for regulating PD-1 expression via promoter modification. Conventional strategies mostly target the coding region: gene knockout completely eliminates PD-1 expression, which can easily induce T cell overactivation and immune imbalance; antibody blockade only exerts a short-term inhibitory effect, lacks tunable quantitative control, and carries a risk of immune-related adverse events. In contrast, promoter modification precisely regulates transcriptional activity, achieving moderate downregulation of PD-1 and alleviating T cell exhaustion, while retaining physiological PD-1 levels, maintaining immune homeostasis, and avoiding adverse reactions caused by overactivation [7]. In addition, this method retains the native structure and function of the PD-1 protein and reduces the risk of protein abnormalities, thus representing a safer and more reasonable regulatory strategy.

B. Structure and Regulatory Elements of the PD-1 Promoter

The PD-1 promoter consists of a core promoter region and an upstream regulatory region that coordinates transcription initiation and efficiency by binding various transcription factors. The core region contains the transcription start site and TATA box, which recruit RNA polymerase and general transcription factors to establish basal PD-1 expression. The upstream region contains multiple cis-acting elements that bind activating or inhibitory transcription factors, enabling dynamic regulation. Minor sequence alterations at these sites can profoundly affect transcription factors' binding and promoter activity, rendering them ideal key targets for PRIME editing [8]. Additionally, epigenetic sites within the PD-1 promoter—such as DNA methylation and histone modification sites—indirectly regulate transcription through chromatin structure changes, forming a complex regulatory network.

C. PRIME Editing Strategies for Regulating Promoters

The core of using PRIME editing to regulate the PD-1 promoter is to introduce precise single-nucleotide modifications that alter the sequence of key regulatory elements, modulate transcriptional activity, and achieve mild downregulation of PD-1 expression. It is specifically divided into core strategies and auxiliary strategies. The core strategy is to target key transcription factor binding sites: through bioinformatics analysis and experimental verification, the key sites where the PD-1 promoter binds to activating transcription factors are screened, and single nucleotide substitutions are made through PRIME editing to reduce its binding efficiency and inhibit transcriptional activity. Simultaneously, binding sites for inhibitory transcription factors can be modified to enhance their binding affinity, enabling dual-directional regulation. This strategy demands precise design to tune the extent of transcriptional repression, balancing the alleviation of T-cell exhaustion with the preservation of immune homeostasis. The auxiliary strategy is to modify epigenetic modification sites: screen key epigenetic sites and change their sequences through PRIME editing, making them prone to inhibitory modifications such as methylation and indirectly reducing transcriptional activity. The two types of strategies work synergistically to achieve quantitative control of PD-1 expression, but it is still necessary to verify the activity and expression level through in vitro experiments and optimize the editing scheme to improve

the regulatory effect.

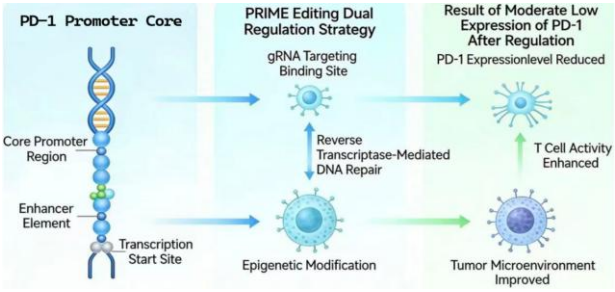

Fig. 1. Schematic illustration of PRIME editing-mediated PD-1 promoter modification.

According to Fig. 1, a schematic illustration of PRIME editing-mediated PD-1 promoter modification. PRIME editing targets the core promoter and enhancer elements of the PD-1 promoter, enabling single-nucleotide precise modification via reverse transcriptase-mediated DNA repair without double-strand breaks. After editing, PD-1 expression is moderately reduced, avoiding complete silencing, while T cell activity is significantly enhanced, improving adaptability and anti-exhaustion capacity in the tumor microenvironment.

D. Delivery System Selection and Optimization

To enable in vitro validation and optimization, an efficient delivery system customized for primary CAR-T cells is essential for PRIME editing-mediated PD-1 promoter modification. The system must accurately deliver the PRIME fusion protein and targeting gRNA while minimizing cytotoxicity and preserving CAR-T cell function. Delivery systems are divided into viral and non-viral vectors. Viral vectors offer high delivery efficiency and stable transfection but pose risks of immunogenicity and random insertion; traditional vectors cannot accommodate large PRIME fusion proteins, requiring backbone modification and packaging optimization [9]. Non-viral vectors are safer and carry no risk of insertional mutagenesis, but electroporation, a conventional method for CAR-T manufacturing, suffers from low efficiency and potential cellular toxicity. Parameter optimization and targeted lipid nanoparticle development are needed to balance safety and editing efficiency.

E. Engineering Preparation and Safety Considerations

Based on an optimized delivery system, engineering preparation of PRIME-edited CAR-T cells must meet rigorous safety and scalability standards. Clinical application relies on standardized protocols and full-process safety control systems to balance editing efficiency, cell function, and translational feasibility. Engineering and safety design focus on four core aspects: process integration, off-target prevention, functional verification, and clinical adaptation. The goal is to integrate PRIME editing seamlessly into standard CAR-T activation and expansion workflows, minimizing extra manipulations and ex vivo processing time while avoiding cellular damage. For safety, off-target effects are prioritized through optimized gRNA design, high-throughput sequencing-based detection, and adjusted editing element dosage. Recent studies have demonstrated that optimized prime editing protocols can achieve editing efficiencies exceeding 78% in primary human cells while maintaining normal cellular function, providing a valuable reference for safety assessment frameworks [10].

IV. Challenge and Future Directions

Despite these advances in cell engineering and manufacturing, the fundamental challenge remains: T-cell exhaustion is the core bottleneck limiting CAR-T efficacy, especially for solid tumor therapy. The current regulatory strategies for PD-1 are divided into two categories, with obvious limitations: PD-1 antibody combination therapy relieves fatigue by blocking inhibitory signals, but requires repeated administration, which can cause systemic immune-related adverse reactions, and the regulatory accuracy is insufficient; knocking out the PD-1 gene through traditional gene editing can achieve permanent expression ablation to relieve inhibition, but it can easily induce T cell overactivation and accelerated depletion, and DNA double-strand breaks can cause genotoxicity. Furthermore, complete loss of PD-1 disrupts immune homeostasis and triggers uncontrolled immune responses. As a next-generation gene editing tool, PRIME editing provides a unique platform for precise modification of the PD-1 promoter owing to its ability to avoid DNA double-strand breaks [6]. By mediating single-nucleotide editing of key promoter regulatory elements, this strategy alleviates T-cell exhaustion while preserving partial PD-1 signaling to maintain immune homeostasis, laying a solid foundation for CAR-T therapy optimization and immunotherapy innovation.

The future development of this strategy can be promoted in multiple directions: first, optimize the technical system by upgrading delivery and engineering, improve editing efficiency and targeting, and elucidate the PD-1 promoter regulatory network through high-throughput analysis for quantitative expression control; second, expand the therapeutic targets by extending promoter modification to other depletion-related factors, and construct multi-target “super CAR-T” cells to enhance their performance in survival and killing capabilities in the solid tumor microenvironment [11]; then, accelerate clinical transformation by promoting preclinical research, conducting trials on refractory solid tumors, and establishing standardized transformation protocols; then, extend the application to other immune-related diseases and provide new immune regulation methods; finally, promote the deep integration of gene editing and immune cell therapy to break through traditional regulatory paradigms, and improve the safety and effectiveness of treatment.

V. Conclusion

This article systematically reviews recent advances in the mechanisms of CAR-T exhaustion, the PD-1 regulatory network, and PRIME editing technology, and draws the following conclusions: T cell exhaustion is the

core obstacle limiting the efficacy of CAR-T, and PD-1, as the core regulator of its exhaustion, is an important target for optimizing CAR-T function. Current PD-1-targeting strategies have inherent limitations, and PRIME editing-mediated promoter modification has significant advantages in safety, accuracy, and function retention, thus providing an innovative path for the construction of universal CAR-T cells with low exhaustion and high persistence. However, the application of PRIME editing in T cells is still in its infancy, and the full complexity of the PD-1 promoter regulatory network remains incompletely understood. Technical optimization can be used to improve the efficiency of PRIME editing, systematically identify the key regulatory elements of the PD-1 promoter, and construct multiple optimized "super CAR-T" cell and apply them in clinical practice. In the future, the precise promoter regulation strategy mediated by PRIME editing can even be extended to other immune checkpoints. It is expected to enable cross-disciplinary applications extending from cancer therapy to other immune-related disorders, including autoimmune diseases and chronic infections, making a significant contribution to the entire field of immunotherapy.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] D. R. Sen, S. A. Wang, O. Rozenblatt-Rosen *et al*., "Epigenetic tuning of PD-1 expression improves exhausted T cell function and viral control," *Nat. Immunol*., vol. 25, no. 10, pp. 1871–1883, 2024.

[2] G. Polcaro, M. Terlizzi, V. Casolaro *et al*., "rs10204525 binding to miR-4717-3d modulates PD-1 expression and predicts the development of immune-related adverse events in patients with advanced cancer treated with anti-PD-1/PD-L1 therapy," *J. Immunother. Cancer*, vol. 13, no. 12, e012207, 2025.

[3] J. Zhao, H. Wu, Z. Sun *et al*., "shRNA-based PD-1 suppression preserves memory phenotype and function of CD19-targeted CAR-T cell," *Transl. Cancer Res*., vol. 14, no. 10, 2025.

[4] A. Khaniya, N. Khuisangeam, S. Tawinwung *et al.*, "A bidirectional EF1 promoter system for armoring CD19 CAR-T cells with secreted anti-PD1 antibodies," *Int. J. Mol. Sci*., vol. 26, no. 23, 11566, 2025.

[5] Z. Jia, J. Wu, J. Zhang, P. Zheng *et al*., "Precision reprogramming in CAR-T cell therapy: Innovations, challenges, and future directions of advanced gene editing," *Int. J. Biol. Sci*., vol. 21, no. 15, pp. 6884–6906, 2025.

[6] K. Petri, E. D'Ippolito, A. Künkele *et al*., "Next-generation T cell immunotherapies engineered with CRISPR base and prime editing: Challenges and opportunities," *Nat. Rev. Clin. Oncol.*, vol. 22, no. 12, pp. 876–891, 2025.

[7] D. Pais Ferreira, S. Shin, V. S. Wacleche *et al*., "Inhibitory PD-1 axis maintains high-avidity stem-like CD8+ T cells," *Nature*, vol. 649, no. 7963, pp. 194–204, 2026.

[8] G. Polcaro, L. Liguori, V. Manzo *et al*., "rs822336 binding to C/EBPβ and NFIC modulates induction of PD-L1 expression and predicts anti-PD-1/PD-L1 therapy in advanced NSCLC," *Mol. Cancer*, vol. 23, no. 1, 63, 2024.

[9] J. Han, X. Wang, Z. Li *et al*., "Unleashing CAR-T potential in solid tumors: Overcoming intrinsic and extrinsic hurdles to improve therapy," Cancer Immunol. Immunother., vol. 75, no. 2, 37, 2026.

[10] R. J. Ji, G. H. Cao, W. Q. Zhao *et al*., "Epitope prime editing shields hematopoietic cells from CD123 immunotherapy for acute myeloid leukemia," *Cell Stem Cell*, vol. 31, no. 11, pp. 1650–1666.e8, 2024.

[11] Y. Chen, X. Zhang, Y. Liu *et al*., "Transcriptional dynamics of CD8+ T-cell exhaustion in immune checkpoint inhibitor resistance at single-cell resolution," *J. Immunother. Cancer*, vol. 13, no. 12, e010892, 2025.