Abstract Number: 58

Epigenetic editing as a promising strategy to enhance efficacy and genomic integrity of CAR T cell therapy

Shutan Jiang, Shaoshuai Mao, Leilei Wu, Yaqin Li, Xiaodong Huang and Bob Zhang, Epigenic Therapeutics, Shanghai, China

Background: Chimeric Antigen Receptor T-cell (CAR-T) therapy has shown remarkable effectiveness and transformational impact in treating hematological malignancies and solid tumors. Site-specific nucleases such as TALENs and CRISPR/Cas9 have been applied in CAR-T therapies to enhance its cellular potency and functions. However, Cas9 cleavage might lead to chromosomal aberrations, raising potential safety issues in CAR-T therapy.

Methods: In this study, we developed epigenetic modulation technology (EPIREG) wherein a DNA cleavage-free domain is fused with epigenetic modulation effectors to manipulate disease-causing genes through the endogenous epigenetic regulation pathway (Figure 1). Utilizing computational biology approach in combination with high-throughput screening, we have optimized and selected single-guide RNAs (sgRNAs) for precise, efficient, and sustainable gene modulations. Then the EPIREG mRNA and sgRNAs were delivered into CAR-T cells using electroporation method (Figure 2).

Results: A proficient method of introducing EPIREG into primary T cells is through electroporation, and when coupled with AI-guided sgRNA, the gene editing efficiency is unparalleled (Figure 3). Delving deeper, sgRNA demonstrates remarkable precision in both individual (Figure 4A) and combined gene (Figure 4B) editing within primary T cells. Consequential to these modifications, CAR-T cells exhibit enhanced CAR expression and stemness potential (Figure 5). *In vitro* examinations further validate the augmented functional attributes of these edited CAR-T cells, evidenced by their increased cytokine secretion and cytotoxic prowess (Figure 6). *In vivo* assessments reveal a significant enhancement in the anti-tumor effectiveness of gene-edited CAR-T cells (Figure 7). These findings collectively herald a transformative phase in CAR-T cell therapeutics driven by EPIREG.

Conclusion: EPIREG demonstrated superior potency, durability, and safety for gene modifications in CAR-T cells. Without Cas9-related risks, EPIREG offers a potential and promising strategy in future CAR-T cell therapies with enhanced efficacy and improved genomic integrity.

Key words: Epigenetics, CAR T cells, RNA, Gene expression, Adoptive immunotherapy

Mechanism of EPIREG in gene regulation

EPIREG's combined effects ensure enduring regulatory influence, achieving tight control over gene accessibility.

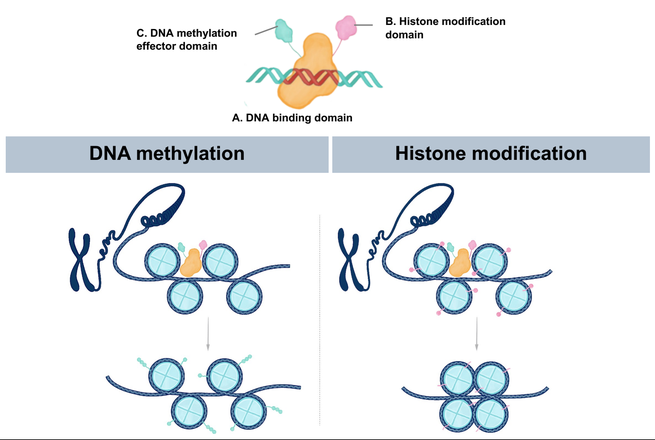

Figure 1. Molecular architecture and functional mechanism of EPIREG
The EPIREG complex is illustrated with three primary domains: (A) DNA binding domain: essential for recognizing specific DNA sequences and anchoring the molecule. (B) Histone modification domain: alters histone structure, influencing gene expression. (C) DNA methylation domain: modifies DNA conformation, affecting gene accessibility. A significant feature of EPIREG's mechanism is its enduring regulatory influence on gene accessibility.

EPIREG significantly augmented CAR-T therapeutic efficacy

EPIREG integration reshapes CAR-T cell therapies, presenting a new paradigm in cancer treatments.

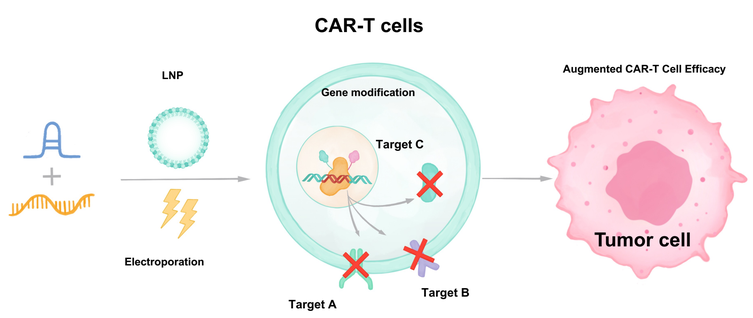

Figure 2. Pioneering the Application of EPIREG in CAR-T Cell Therapies
This illustration highlights the role of EPIREG mRNA in CAR-T cell therapies, complemented by co-delivery of sgRNAs. Key stages include electroporation: introducing EPIREG mRNA and sgRNAs into CAR-T cells. Lipid Nanoparticle (LNP) Delivery: encapsulating and transporting EPIREG mRNA and sgRNAs for optimal uptake. Action of EPIREG: modulating cellular pathways in CAR-T cells, influencing therapeutic trajectory. Augmented CAR-T cell efficacy: increased therapeutic potency of CAR-T cells post-modulation by EPIREG.

Efficient Electroporation and Gene Editing of EPIREG

Electroporation efficiently delivers EPIREG, and AI-predicted sgRNAs ensure optimal gene editing.

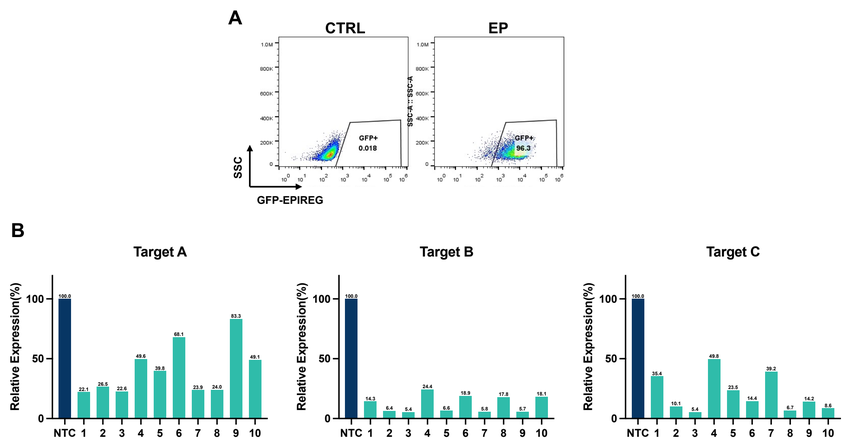

Figure 3. Efficient Electroporation of EPIREG into Human Primary T Cells and Subsequent Gene Editing Efficiency Analysis.
This figure illustrates the process of EPIREG introduction into human T cells and sgRNA editing efficiency evaluation. (A) Efficient electroporation: demonstrating successful EPIREG introduction using the electroporation method. (B) Gene editing efficiency with AI-predicted sgRNAs: targeting three genes, with AI-predicted sgRNA sequences evaluated for editing efficiency post-electroporation.

Detailed investigation of sgRNA's gene editing capabilities in T cells

The chosen sgRNA exhibits profound efficacy in both single and multi-gene editing, paving the way for advanced gene therapies.

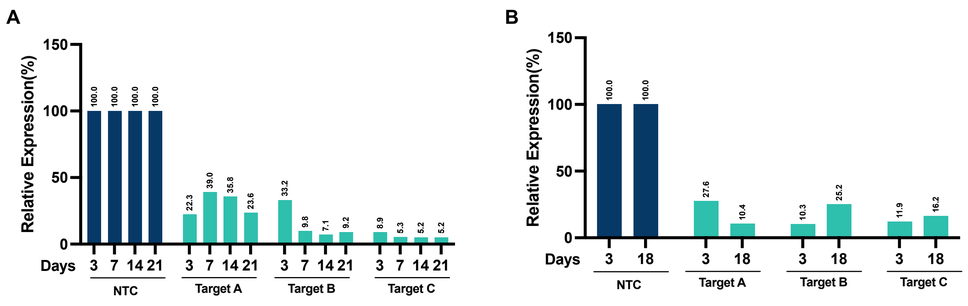

Figure 4. Precision Single and Multi-Gene Editing in Primary T Cells
This figure delves into sgRNA-mediated gene editing in primary T cells. (A) Single gene editing: analyzing targeted modifications on genes with consistent editing effects across intervals. (B) Simultaneous multi-gene editing: concurrent editing of three genes, assessing co-editing efficiency.

EPIREG modulation enhanced functions of CAR-T cells

Precise gene editing amplifies CAR-T cells' stemness and CAR expression, hinting at advanced therapeutic strategies.

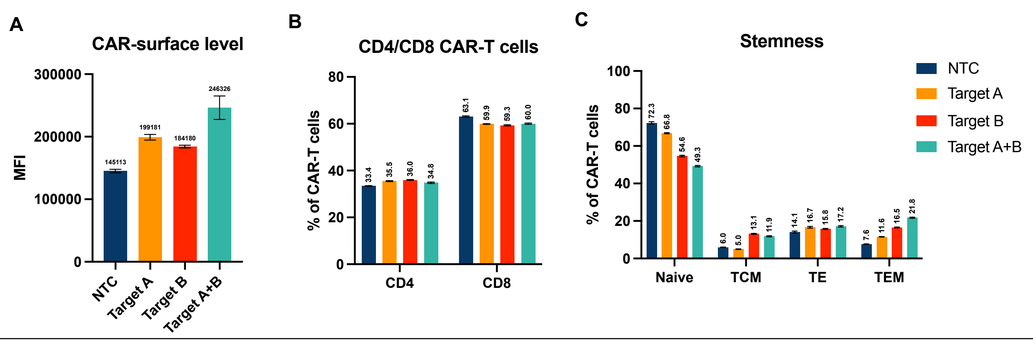

Figure 5. Enhanced Stemness and CAR Expression in CAR-T Cells Following Targeted Gene Editing
This figure explores the outcomes of targeted gene edits in CAR-T cells. (A) CAR molecule expression: upregulated CAR expression post-editing with enhanced effects from multi-target edits. (B) $CD4^+$ to $CD8^+$ T cell ratio: analysis post-editing indicates stable T cell subtype ratios. (C) Stemness assessment: increased proportion of stem-like cells in edited CAR-T cell groups.

EPIREG enhanced *in vitro* Functions of CAR-T cells

Gene-edited CAR-T cells exhibit enhanced cytokine secretion and cytotoxicity *in vitro*.

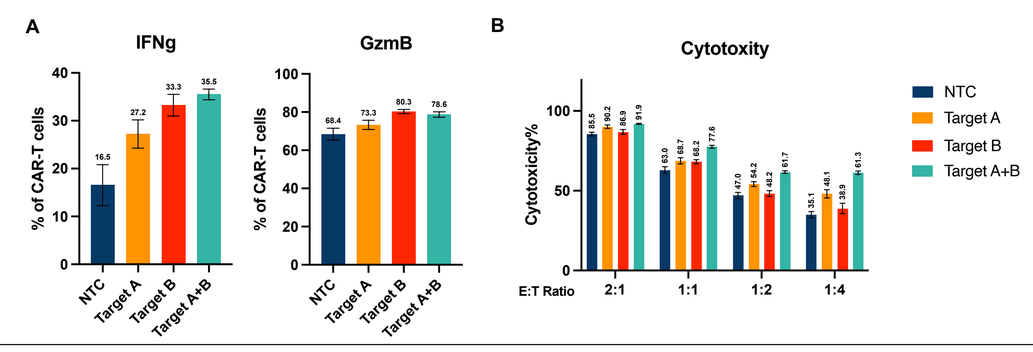

Figure 6. Functional Enhancement of Gene-Edited CAR-T Cells in *in vitro* Assessments.
This figure evaluates the in vitro functionalities of gene-edited CAR-T cells. (A) Cytokine secretion: enhanced cytokine secretion by CAR-T cells post-editing. (B) *in vitro* cytotoxicity: increased cytotoxic potential of CAR-T cells after gene editing.

EPIREG enhanced *in vivo* Anti-Tumor Efficacy of CAR-T Cells

in vivo results robustly demonstrate the enhanced anti-tumor potency of gene-edited CAR-T cells through size assessments and statistical validation.

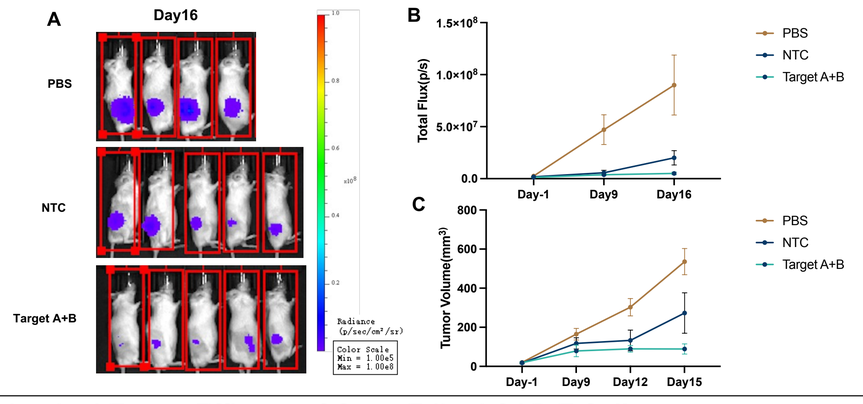

Figure 7. *in vivo* Anti-Tumor Efficacy of Gene-Edited CAR-T Cells
This figure highlights the *in vivo* anti-tumor efficacy of gene-edited CAR-T cells. (A) Tumor size imaging at Day 16: captures tumor size changes post CAR-T cell injection using live imaging (IVIS). (B) Statistical analysis: reveals enhanced anti-tumor effects in the dual-gene edited CAR-T group compared to the NTC group. (C) Tumor size data: provides quantitative evidence, supporting the observations in panels A and B, about the improved anti-tumor efficacy.

Summary

1. CAR-T therapy, transformative for malignancies, is enhanced by EPIREG, an epigenetic solution that addresses Cas9-induced chromosomal aberrations without DNA cleavage.
2. Combined with AI-optimized sgRNAs, EPIREG boosts gene modulation precision and efficiency.
3. Through electroporation, EPIREG is proficiently introduced into T cells, leading to CAR-T cells with elevated CAR expression and stemness.
4. The enhanced CAR-T cells showcase increased *in vitro* functionality and *in vivo* anti-tumor potency.
5. EPIREG stands out as a potent, durable, and safer strategy for the future of CAR-T cell therapies.

Acknowledgements

We thank the entire Epigenic Therapeutics team and our collaborators.

Contact Email Address: bob.zhang@epigenictx.com
Website of EPIGENICTX: www.epigenictx.com