58 EPIGENETIC EDITING AS A PROMISING STRATEGY TO ENHANCE EFFICACY AND GENOMIC INTEGRITY OF CAR T CELL THERAPY

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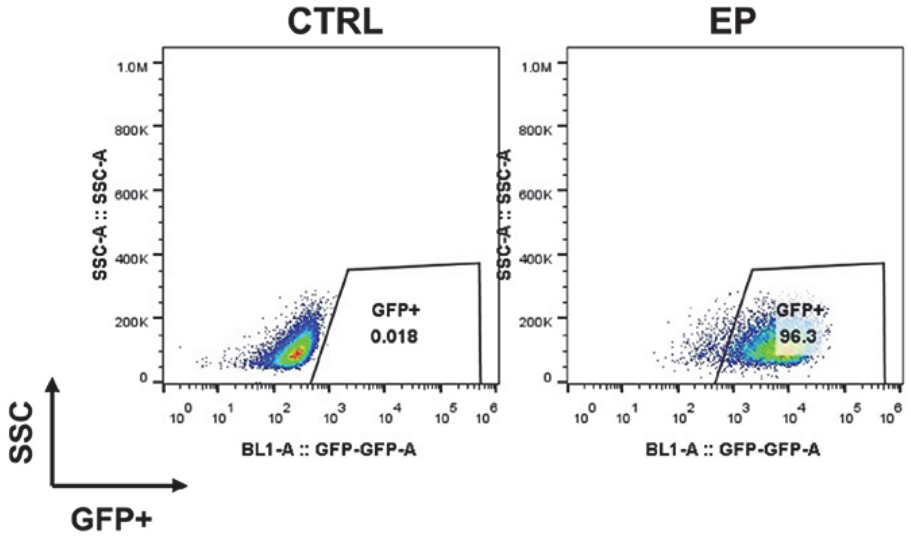
Background Chimeric Antigen Receptor T-cell (CAR-T) therapy has shown remarkable effectiveness and transformational impact in treating hematological malignancies and solid tumors. Site-specific nucleases such as TALENs and CRISPR/Cas9 have been applied in CAR-T therapies to enhance its cellular potency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

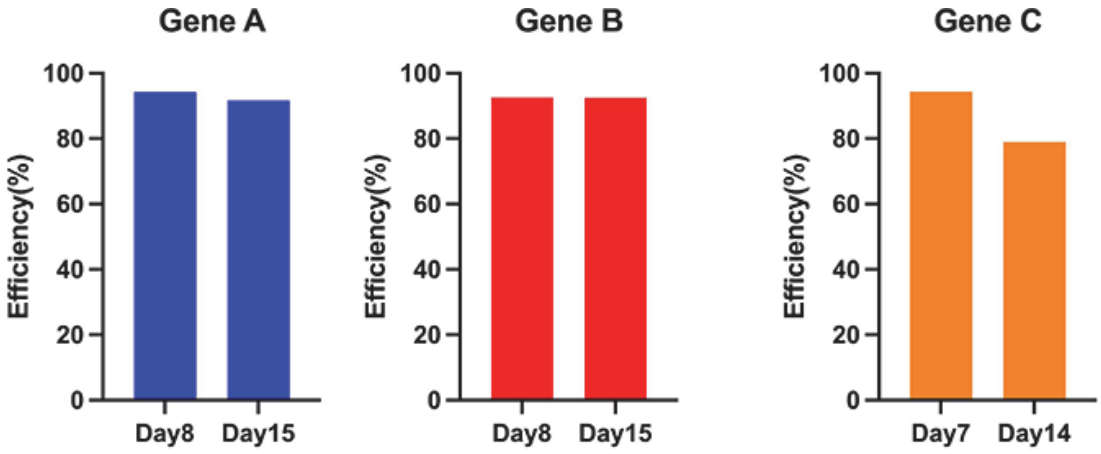
Methods In this study, we developed epigenetic modulation technology (EPIREG) wherein a DNA cleavage-free domain is fused with epigenetic modulation effectors to manipulate disease-causing genes through the endogenous epigenetic regulation pathway. Utilizing computational biology approach in combination 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.

Results Delivery efficiency into CAR-T cells can reach over 96% (shown in figure 1), while maintaining high cell viability. Consequently, a high level of gene silencing (up to 90% for target gene A, B, and C, respectively, shown in figure 2) was observed. Furthermore, this gene silencing effect was sustained for more than 2 weeks, sufficient manufacturing time for CAR-T cell products. EPIREG also showed capabilities to simultaneously manipulate multiple target genes (efficiency shown in figure 3), thereby strengthening the performance of CAR-T cells. These cells with multiplex gene modifications displayed improved cytotoxicity, increased cytokine production, and sustained presence both *in vitro* and *in vivo*. Importantly, EPIREG demonstrated minimal off-target effects and circumvented the risks of chromosomal aberrations associated with Cas9.

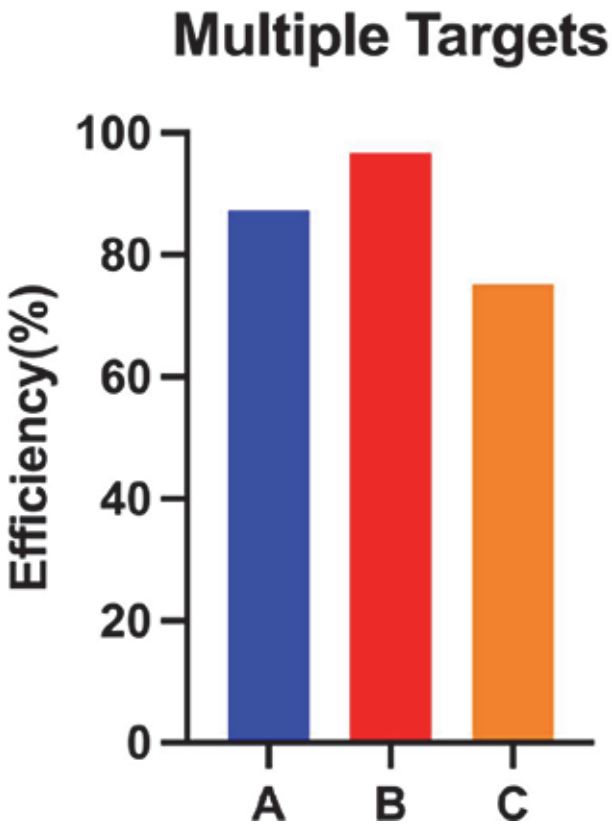
Conclusions EPIREG demonstrated superior potency, durability, and safety for gene modifications in CAR-T cells. Without Cas9-related risks, EPIREG offers a potential and promising strategy in future CAR-T cell therapies with enhanced efficacy and improved genomic integrity.



Abstract 58 Figure 1 Efficient electroporation of EPIREG mRNA into T cells.



Abstract 58 Figure 2 EPIREG achieves efficient and sustained editing effects on multiple targets.



Abstract 58 Figure 3 EPIREG enables simultaneous and efficient editing of three targets.

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