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CRISPR in Immuno-Oncology: Engineering CAR-T and Next-Generation Immune Cells

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Abstract

Immuno-oncology has reshaped the therapeutic landscape of cancer treatment by shifting focus from directly targeting tumor cells to mobilizing the immune system against malignancies. Among the most transformative advances in this field is the development of chimeric antigen receptor T-cell therapy, which has demonstrated remarkable efficacy in hematologic cancers. However, persistent challenges such as limited durability, immune escape, toxicity, and poor performance in solid tumors have constrained its broader clinical impact. The emergence of clustered regularly interspaced short palindromic repeats (CRISPR) genome editing has introduced a powerful and versatile platform for engineering immune cells with enhanced specificity, persistence, and functionality. CRISPR-based approaches enable precise gene knockout, targeted gene insertion, epigenetic modulation, and multiplex editing, allowing researchers to redesign immune cells at multiple regulatory levels. These capabilities have significantly advanced CAR-T cell engineering and have catalyzed the development of next-generation immune effectors, including natural killer cells, macrophages, and stem cell-derived immune populations. Furthermore, CRISPR technology has opened new avenues for overcoming the immunosuppressive tumor microenvironment, improving safety profiles, and enabling scalable, off-the-shelf therapies. This review provides a comprehensive examination of CRISPR applications in immuno-oncology, with an emphasis on CAR-T optimization and the engineering of next-generation immune cells. It discusses mechanistic foundations, technological innovations, preclinical and clinical advancements, safety considerations, and future directions. Collectively, CRISPR-driven immune engineering represents a paradigm shift toward more precise, effective, and accessible cancer immunotherapies.

Keywords: CRISPR, CAR-T cells, immuno-oncology, genome editing, cancer immunotherapy, T-cell engineering, NK cells, tumor microenvironment, multiplex editing, base editing

1. Introduction

The treatment of cancer has undergone a profound transformation over the past several decades, evolving from non-specific cytotoxic therapies to highly targeted and biologically informed strategies [1, 2]. Traditional approaches such as chemotherapy and radiation therapy [3], while effective in certain contexts, are often associated with significant toxicity and limited selectivity. The advent of targeted therapies improved specificity by focusing on molecular drivers of tumor growth, yet resistance

mechanisms frequently emerged, explaining the need for more adaptable and durable treatment modalities. Immuno-oncology represents a paradigm shift in cancer treatment [4], emphasizing the ability of the immune system to recognize and eliminate malignant cells. Central to this approach is the concept of immune surveillance, whereby immune cells continuously monitor tissues for abnormal or transformed cells [5, 6]. However, tumors often develop sophisticated mechanisms to evade immune detection, including downregulation of antigen presentation, secretion of immunosuppressive factors, and induction of inhibitory signaling pathways [7, 8].

Among the most successful immunotherapeutic strategies to date is CAR-T cell therapy, which involves genetically modifying a patient's T cells to express synthetic receptors that target tumor-specific antigens [9, 10]. These engineered cells are expanded ex vivo and reinfused into the patient, where they can recognize and destroy cancer cells with high specificity [11, 12]. Clinical successes in diseases such as acute lymphoblastic leukemia and certain lymphomas have validated this approach and led to regulatory approvals of multiple CAR-T products [13, 14]. Despite these achievements, CAR-T therapy faces significant limitations. One of the primary challenges is the heterogeneity of tumors, which can lead to antigen escape and treatment resistance [15]. Additionally, CAR-T cells often exhibit limited persistence in vivo, reducing their long-term efficacy [16]. The tumor microenvironment further complicates treatment by creating conditions that suppress immune activity, including hypoxia, nutrient deprivation, and the presence of regulatory immune cells.

The integration of CRISPR genome editing into immuno-oncology has provided a powerful solution to many of these challenges. CRISPR technology allows for precise and efficient modification of the genome, enabling researchers to reprogram immune cells with unprecedented control [17]. Unlike earlier gene editing tools, CRISPR systems are highly adaptable and capable of targeting multiple genes simultaneously, making them particularly well suited for complex biological systems such as immune cells [18].

In the context of CAR-T therapy, CRISPR has been used to enhance antitumor activity by disrupting inhibitory pathways, improving cell persistence, and enabling the creation of universal donor cells [19]. Beyond CAR-T cells, CRISPR is facilitating the development of alternative immune cell therapies, including NK cells and macrophages [20], which offer distinct advantages in targeting solid tumors and reducing treatment-related toxicities. As the field continues to evolve, CRISPR-based immune engineering is poised to play a central role in the next generation of cancer therapies [21]. By combining advances in genome editing, synthetic biology, and immunology, researchers are developing increasingly sophisticated approaches to overcome the limitations of current treatments and improve patient outcomes.

2. Molecular Foundations of CRISPR Systems in Immune Engineering

The success of CRISPR in immuno-oncology is rooted in its molecular simplicity and versatility. At its core, the CRISPR-Cas system consists of two main components: a guide RNA that determines the target sequence and a Cas nuclease that performs the DNA cleavage. The most commonly used nuclease, Cas9, introduces a double-strand break at the target site, which is subsequently repaired by the cell's endogenous DNA repair machinery. Two primary repair pathways are involved in this process. Non-homologous end joining is an error-prone mechanism that often results in insertions or deletions at the

break site, leading to gene disruption [22]. This pathway is commonly used to knock out genes that inhibit immune function, such as checkpoint receptors. Homology-directed repair, on the other hand, allows for precise insertion of new genetic material when a donor template is provided [23]. This mechanism is particularly useful for inserting CAR constructs into specific genomic loci.

The specificity of CRISPR targeting is determined by the guide RNA sequence, which binds to the complementary DNA sequence adjacent to a protospacer adjacent motif [24]. The requirement for this motif ensures that the Cas nuclease only cleaves DNA at appropriate locations. Advances in guide RNA design and the development of high-fidelity Cas variants have significantly improved targeting accuracy, reducing the risk of off-target effects [25]. Beyond traditional CRISPR-Cas9 editing, a range of modified systems has been developed to expand the functionality of genome editing. Base editors, for example, enable the direct conversion of one nucleotide to another without introducing double-strand breaks [26]. This approach reduces the likelihood of unintended genomic alterations and is particularly useful for correcting point mutations or fine-tuning gene function.

Prime editing represents another major advancement, allowing for precise insertion, deletion, or substitution of DNA sequences using a reverse transcriptase fused to a Cas nickase [27]. This technology offers greater flexibility and precision than traditional CRISPR approaches and holds significant promise for therapeutic applications. CRISPR interference and CRISPR activation systems provide additional tools for regulating gene expression without altering the underlying DNA sequence. By using catalytically inactive Cas proteins fused to transcriptional regulators, researchers can either repress or activate target genes. These systems enable dynamic control of gene expression, which is particularly valuable in immune cells that must respond to changing environmental conditions. The integration of these diverse CRISPR-based tools into immuno-oncology has created a powerful platform for engineering immune cells with tailored properties. By combining gene knockout, targeted insertion, and gene regulation, researchers can design complex genetic circuits that enhance antitumor activity, improve persistence, and reduce toxicity.

3. Engineering CAR-T Cells with CRISPR Precision

The application of CRISPR technology to CAR-T cell engineering has significantly advanced the field by enabling more precise and controlled genetic modifications. Traditional CAR-T cell generation relies on viral vectors to introduce CAR constructs into T cells [28, 29]. While effective, this approach results in random integration of the transgene, leading to variability in expression levels and potential risks associated with insertional mutagenesis. CRISPR-based strategies address these limitations by enabling targeted integration of CAR constructs into specific genomic loci [30]. One commonly used site is the T-cell receptor alpha constant (TRAC) locus, where insertion of the CAR transgene simultaneously disrupts the endogenous T-cell receptor and ensures uniform expression of the CAR. This approach enhances the functionality of CAR-T cells and reduces the risk of graft-versus-host disease.

In addition to targeted insertion, CRISPR is widely used to disrupt genes that negatively regulate T-cell activity [31]. The programmed cell death protein 1 (PD-1) pathway is a key inhibitory mechanism that limits T-cell responses in the tumor microenvironment [32]. By knocking out PD-1, CRISPR-engineered CAR-T cells can maintain their activity even in the presence of immunosuppressive signals. Multiplex editing represents one of the most powerful applications of CRISPR in CAR-T engineering. By

simultaneously targeting multiple genes, researchers can create cells with enhanced antitumor properties. For example, combining PD-1 knockout with deletion of other inhibitory receptors such as LAG-3 or TIM-3 can further improve T-cell function [33]. Additionally, inserting genes that promote cell survival or resistance to metabolic stress can enhance persistence and efficacy. CRISPR also enables the development of universal CAR-T cells that can be used across multiple patients. By removing endogenous T-cell receptors and major histocompatibility complex molecules, these cells can evade immune rejection and reduce the risk of alloreactivity [34]. This approach has the potential to significantly reduce the cost and complexity of CAR-T therapy by enabling the production of off-the-shelf products.

4. Reprogramming the Tumor Microenvironment Through CRISPR-Engineered Immune Cells

One of the most persistent barriers to effective immunotherapy is the tumor microenvironment, a highly complex and dynamic ecosystem that actively suppresses immune responses [35, 36]. While early successes in CAR-T therapy were largely confined to hematologic malignancies, the extension of these therapies to solid tumors has revealed the profound influence of the tumor microenvironment on therapeutic outcomes [37, 38]. This environment is characterized by hypoxia, acidic pH, nutrient deprivation, dense stromal architecture, and a network of immunosuppressive cells including regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages.

CRISPR technology has provided an unprecedented opportunity to reprogram immune cells to function effectively within this hostile environment. One of the most widely explored strategies involves disrupting signaling pathways that mediate immunosuppression [39, 40]. Transforming growth factor beta is a critical immunosuppressive cytokine present in many tumors, capable of inhibiting T-cell proliferation and effector function [41]. By using CRISPR to knock out the TGF-β receptor in T cells, researchers have demonstrated enhanced resistance to suppressive signaling, allowing engineered cells to maintain activity even in highly inhibitory conditions [42-44].

Tumor cells consume large amounts of glucose and amino acids, creating a metabolically constrained environment for immune cells [45]. CRISPR-based modifications can enhance metabolic fitness by upregulating pathways involved in oxidative phosphorylation or glycolysis, thereby enabling T cells to sustain their activity despite nutrient limitations [46, 47]. These metabolic interventions are increasingly recognized as essential components of next-generation immunotherapy design. CRISPR engineering also allows immune cells to actively reshape the tumor microenvironment rather than merely resist it. For example, engineered T cells can be programmed to secrete pro-inflammatory cytokines such as interleukin-12 or interferon gamma, which enhance antigen presentation and recruit additional immune effectors [48]. This approach effectively transforms the tumor microenvironment from an immunosuppressive niche into an immune-active site.

5. CRISPR Engineering of Natural Killer Cells

Natural killer cells represent a promising alternative to T-cell-based therapies due to their innate ability to recognize and eliminate tumor cells without prior sensitization [49]. Unlike T cells, NK cells do not rely on antigen presentation through major histocompatibility complex molecules, which are often downregulated in tumors as a mechanism of immune escape. This unique property makes NK cells

particularly attractive for targeting a broad range of cancers. CRISPR technology has significantly advanced the engineering of NK cells by enabling precise modifications that enhance their cytotoxic activity, persistence, and resistance to suppression [50]. One of the primary challenges in NK cell therapy has been their relatively short lifespan in vivo, through CRISPR-mediated insertion of cytokine support genes such as interleukin-15, researchers have been able to extend NK cell survival and improve their antitumor efficacy [51, 52].

Another important application of CRISPR in NK cell engineering is the disruption of inhibitory receptors. Similar to T cells, NK cells express checkpoint molecules that can limit their activity in the tumor microenvironment [53]. By knocking out genes encoding these receptors, CRISPR-enhanced NK cells can maintain a more sustained and robust response against cancer cells [54]. CRISPR also facilitates the introduction of chimeric antigen receptors into NK cells, creating CAR-NK therapies that combine the specificity of CAR targeting with the innate cytotoxicity of NK cells [55-57]. These therapies offer several advantages over CAR-T cells, including a lower risk of cytokine release syndrome and the potential for off-the-shelf use. Early clinical studies have demonstrated encouraging safety profiles and antitumor activity, suggesting that CAR-NK cells may play a significant role in the future of immuno-oncology [58, 59]. Furthermore, NK cells are less prone to causing graft-versus-host disease, making them ideal candidates for allogeneic therapies [60]. CRISPR editing can further enhance their compatibility by removing residual immunogenic markers, enabling the development of universal donor NK cell products [61, 62]. This scalability addresses one of the major logistical challenges associated with personalized CAR-T therapies.

6. Macrophage Engineering and Innate Immune Reprogramming

While T cells and NK cells have dominated the landscape of immunotherapy, macrophages are emerging as powerful players in cancer treatment due to their ability to infiltrate tumors and modulate immune responses [63, 64]. Tumor-associated macrophages often adopt an immunosuppressive phenotype that supports tumor growth and metastasis [65]. Reprogramming these cells into pro-inflammatory, tumor-fighting effectors represents a promising therapeutic strategy. CRISPR technology enables precise manipulation of macrophage polarization by targeting genes that regulate their functional state [66]. By disrupting pathways associated with immunosuppressive phenotypes, such as those mediated by STAT3 or IL-10 signaling, researchers can shift macrophages toward a pro-inflammatory profile that enhances tumor clearance [67].

Engineered macrophages can also be designed to express chimeric antigen receptors, creating CAR-macrophages that combine antigen specificity with phagocytic activity. Unlike CAR-T cells, which primarily kill tumor cells through cytotoxic mechanisms, CAR-macrophages can engulf and digest cancer cells while simultaneously presenting tumor antigens to T cells, thereby bridging innate and adaptive immunity [68, 69]. CRISPR-based approaches also allow macrophages to secrete cytokines and chemokines that recruit and activate other immune cells [70]. This coordinated response can amplify the overall antitumor effect and overcome localized immunosuppression. Additionally, macrophages can penetrate dense tumor stroma more effectively than T cells, making them particularly useful in treating solid tumors. The integration of CRISPR into macrophage engineering is still in its early stages, but preclinical studies have demonstrated promising results. As delivery methods improve and our

understanding of macrophage biology deepens, these cells are likely to become an important component of next-generation immunotherapies.

7. Multiplex Editing and Synthetic Immune Circuit Design

One of the defining advantages of CRISPR technology is its ability to perform multiplex editing, allowing simultaneous modification of multiple genes within a single cell. This capability is particularly valuable in immuno-oncology, where complex networks of signaling pathways regulate immune function and tumor interactions. Multiplex editing enables the creation of highly sophisticated immune cells that can overcome multiple barriers to effective therapy [71]. For example, a single CAR-T cell can be engineered to lack inhibitory receptors, express a CAR construct, secrete supportive cytokines, and resist metabolic stress [72, 73]. These multi-layered modifications significantly enhance the overall therapeutic potential of engineered cells.

Beyond simple gene editing, CRISPR is also being used to design synthetic gene circuits that enable dynamic and context-dependent responses. These circuits can be programmed to activate or deactivate specific functions based on environmental cues, such as the presence of tumor antigens or inflammatory signals. This level of control allows for more precise targeting and reduces the risk of off-target effects. For instance, logic-gated CAR-T cells can be engineered to require the presence of multiple antigens before activation, reducing the likelihood of attacking healthy tissues [74, 75]. Similarly, inducible systems can be designed to regulate cytokine production, minimizing the risk of cytokine release syndrome. The development of these advanced genetic circuits represents a convergence of synthetic biology and immuno-oncology. CRISPR serves as the foundational tool that enables the precise construction of these systems, paving the way for increasingly intelligent and adaptive therapies.

8. Delivery Systems for CRISPR in Immune Cells

Efficient delivery of CRISPR components into immune cells is a critical factor in the success of genome editing. Several delivery methods have been developed, each with its own advantages and limitations. Viral vectors, such as lentiviruses and adeno-associated viruses, have been widely used due to their high efficiency [76]. However, concerns about insertional mutagenesis and immunogenicity have prompted the development of alternative approaches. Non-viral delivery methods, particularly electroporation of CRISPR ribonucleoprotein complexes, have gained popularity in immune cell engineering [77, 78]. This approach involves introducing pre-formed Cas protein and guide RNA directly into cells, resulting in transient expression and reduced risk of off-target effects. Electroporation is particularly well suited for ex vivo editing of T cells and NK cells [79]. Lipid nanoparticles and other nanocarrier systems are also being explored for in vivo delivery of CRISPR components [80]. These technologies offer the potential to edit immune cells directly within the body, eliminating the need for complex ex vivo manipulation. While still in early stages of development, these approaches could significantly expand the accessibility of CRISPR-based therapies. The choice of delivery method depends on the specific application and desired outcomes. Ongoing research is focused on improving delivery efficiency, reducing toxicity, and enabling targeted editing of specific cell populations.

9. Future Perspectives in CRISPR-Driven Immuno-Oncology

The future of CRISPR in immuno-oncology is defined by increasing precision, integration, and adaptability. As genome editing technologies continue to evolve, the ability to design highly specialized immune cells tailored to individual patients and tumor types will become increasingly feasible. One of the most promising directions is the combination of CRISPR with other therapeutic modalities, such as checkpoint inhibitors, oncolytic viruses, and personalized vaccines. These combination approaches have the potential to address multiple aspects of tumor biology simultaneously, improving overall treatment outcomes.

Advances in delivery technologies will also play a critical role in expanding the reach of CRISPR-based therapies. In vivo editing approaches, which allow for direct modification of immune cells within the body, could simplify treatment and reduce costs. However, achieving precise and targeted delivery remains a significant challenge. Another important area of development is the use of multi-omics data to guide therapy design. By integrating genomic, transcriptomic, and proteomic information, researchers can gain a more comprehensive understanding of tumor-immune interactions and identify optimal targets for intervention. Ultimately, the continued success of CRISPR in immuno-oncology will depend on the ability to balance innovation with safety and accessibility. As the field matures, collaborative efforts across disciplines will be essential for translating scientific advances into clinical benefits.

Conclusion

CRISPR technology has ushered in a new era in immuno-oncology, enabling precise and versatile engineering of immune cells for cancer therapy. From enhancing CAR-T cell performance to developing next-generation immune effectors, CRISPR has expanded the possibilities of what can be achieved in cancer treatment. While challenges related to safety, delivery, and regulation remain, ongoing advancements are steadily addressing these issues. The integration of CRISPR with emerging technologies such as artificial intelligence, synthetic biology, and advanced delivery systems is accelerating the development of more effective and personalized therapies. As these innovations continue to evolve, CRISPR-driven immune engineering is poised to become a cornerstone of modern oncology, offering new hope for patients with diverse and challenging malignancies.

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