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The Feasibility of Using CRISPR-Cas9 to Knock Out IL-6 Receptor in CAR-T Cells to Mitigate Cytokine Release Syndrome in Cancer Therapy

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Abstract: Cytokine release syndrome (CRS) is a common complication associated with chimeric antigen receptor T cell (CAR-T) therapy, primarily driven by excessive interleukin-6 (IL-6) production. Although the use of IL-6 inhibitors and corticosteroids can partially alleviate CRS, these approaches often yield limited success and may compromise CAR-T cell functionality. Recent advancements in gene-editing technologies, particularly CRISPR-Cas9, offer significant opportunities by enabling site-specific knockout of the IL-6 receptor (IL-6R) in CAR-T cells. In vitro studies have demonstrated that IL-6 gene deletion substantially reduces CRS-related inflammatory responses while preserving the anti-tumor capabilities of CAR-T cells. This strategy not only addresses inflammation at its source but also mitigates harmful systemic inflammatory responses, thereby improving therapeutic outcomes. This review highlights the pivotal role of IL-6 in CRS pathophysiology, the shortcomings of conventional treatment strategies, and the potential of genome-editing approaches such as IL-6R knockout. By precisely targeting the root causes of IL-6-mediated inflammation, this approach aims to optimize both the efficacy and safety of CAR-T cell therapy.

Keywords: CAR-T therapy, cytokine release syndrome, IL-6 receptor, CRISPR-Cas9, immunotherapy.

1. Introduction

The development of CAR-T therapy has shaken up the field of modern cancer treatment by bringing important changes in managing hematological malignancies [1]. A hallmark feature that determines its outstanding therapeutic effect is also a major limitation: the relapsing CRS that is severe and can be fatal [2]. CRS occurs as a result of excessive immune system cell activation, triggered by the overproduction of cytokines, continuing the systemic inflammatory response [3]. Clinical symptoms of CRS fall into the following categories: milder ones can be fevers and fatigue, while the more relevant ones are represented by multiple organ failures [4]. The management of CRS syndrome currently involves IL-6 inhibitors, like tocilizumab, which are recommended as the best standard treatment for CRS [1, 2]. These therapeutic agents have some adverse effects and show inconsistent efficacy with adverse effects, particularly in severe forms of CRS [5].

The advent of genome editing technology, and CRISPR-Cas9 in particular, could be seen as a key turning point for addressing CRS in the near future [6]. By knocking out IL-6R in CAR-T, this

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mechanism may disable the cytokines transduction which causes CRS, and also keep the anti-tumor function of CAR-T cells [3].

Recent trials have demonstrated gene silencing of IL-6 as an effective strategy for stopping the augmented secretion of cytokine, which alleviates CRS-related toxicity, thus favoring the recovery and survival of patients [4, 6]. However, studies mainly focused on the preliminary validations of inflammation control, lacking further research into the mechanisms of action [4]. Additionally, people have limited awareness of the potential adverse effects of evaluating the safety and feasibility of the translations [4, 6]. Particularly, the potential impact on CAR-T cells' long-term efficacy and safety also raises concerns about the safety and feasibility of this approach [4, 6].

In view of these imperfect places, this review proposes an additional possibility of using CRISPR-Cas9 for IL-6R to get knocked off in CAR-T cells. The described approach aims to fundamentally suppress IL-6-mediated cytokine release, not only alleviating CRS but also theoretically preserving the anti-tumor efficacy of CAR-T cells, thereby offering a new direction to address these research challenges.

2. The Role of IL-6 in CRS

CRS is a frequently observed adverse effect of CAR-T cell therapy, which is marked by widespread inflammation triggered by the excessive release of cytokines. Among these cytokines, IL-6 plays a key role in the development of CRS. IL-6 is a multifunctional cytokine produced within a synapse as a result of T cell, macrophage cell, and other immune cells activation during immune response [3]. In CAR-T therapy, CAR-T cells stimulate the immune response during target recognition and killing [7]. Accessory cells in the immune system release many cytokines like IL-6 afterward [7]. IL-6 binds to IL-6R, which starts a cascade of inflammatory reactions, and results in acute-phase protein generation, vessel dilatation, and immune cell infiltration [8]. The disproportionate activity of IL-6 induced by the overactivation leads to CRS symptoms like fever, organ dysfunction, and very complex conditions too [2].

In CAR-T therapy-induced CRS, a variety of disease severity was related to high levels of IL-6 [9]. The observed correlation proved that IL-6 might be one of the most important pro-inflammatory cytokines in CRS [6]. The hyperactivation of IL-6 was identified as a key pathogenic mechanism that leads to the syndrome, making it an attractive target for therapeutic platforms to alleviate CRS-related complications [1].

2.1. Current Approaches and Limitations: Tocilizumab and Corticosteroids

The primary strategies for managing CRS include tocilizumab, a monoclonal antibody targeting IL-6R, and corticosteroids such as dexamethasone [1, 2]. The major reason for tocilizumab's effectiveness in alleviating CRS symptoms is because it disrupts obviously the source of IL-6, leading to lower levels of IL-6 in the systemic circulation, which will help to improve the clinical outcome in severe CRS cases [7]. Here, however, it is limited to an intervention with exogenous [5]. Its application could come only after the initialing of one of CAR-T cell-mediated IL-6 overproduction [5]. In addition, IL-6 gene expression plays a pivotal role in the differentiation, proliferation, and survival of CAR-T cells [8]. Inhibition of IL-6 signaling could obviously have unwanted consequences, like impairment of CAR-T cell expansion, reduction of CAR-T cell persistence, and drop in the CAR-T cells-to-tumor killing efficacy [1, 8]. Thus, these limitations underscore the need for a platform of research to find new approaches that can both mitigate the CRS and keep the CAR-T cells functional.

Additionally, corticosteroids become one of the key players in the strategy of dampening cytokine storm, and their common representatives are dexamethasone [2]. To some extent, however, there are

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some risks as T-cell functions are inhibited which leads to the reduction of T-cell activation, rapid division, and survival, which are imperative for CAR-T to persist [1]. This blockade can be manifested by the level of downregulation of IL-6 production, impaired T cell immune response expansion, and increased apoptosis, thereby leading to the weakening of the therapeutic efficacy of CAR-T cells [1]. The existence of already imbalanced inflammatory processes under CRS, results in decreased immune functions such as the T cell allergic response, leading to secondary bacterial infections, that are potentially hazardous for clinical practice [1, 2]. Notably, corticosteroids, such as tocilizumab, do not correct the cause of CRS, considered the reason IL-6 is overproduced by CAR-T cells themselves [6]. They work as protocols implemented after CRS manifestation by themselves instead of preventive procedures that would avoid the whole event.

These constraints highlight the necessity of looking for other targeted interventions, such as genetic approaches, which reduce the mechanisms perpetuating IL-6-dependent inflammation and do not mar the efficiency of CAR-T cells.

2.2. CRISPR-Cas9 and IL-6R Knockout

The deficiencies of the current CRS management of the methods ratify the demand for much more accurate and advanced ones [1]. Progressions in genome editing technologies, particularly the CRISPR-Cas9 system, have enabled new approaches to fight such challenges at the molecular level [6]. One approach that seems promising is utilizing CRISPR-Cas9 to knock out IL-6R on CAR-T cells specifically, making the CAR-T cells incapable of responding to the cytokine signaling pathways that lead to CRS [3]. Such modification may potentially alleviate IL-6-induced inflammatory signaling to the dispatched CAR-T cells, thus lowering systemic inflammation fixed to CRS [8]. Even though this technique has not yet reached its full potential, it remains promising for being the most useful method for improving CAR-T safety without diminishing anti-tumor efficacy [10].

3. Experimental Evidence Supporting IL-6 Targeting in CRS Management

Modern-day experimental studies highlight the pivotal position of IL-6 in the pathogenesis of CRS in the last few years, and researchers still searching for novel strategies based on IL-6-related pathways to mitigate its effects [8]. These studies not only demonstrate the inadequacy of current treatment methods but also provide a good reason to believe that the strategy of knocking out IL-6R on the molecular level could be a reasonable approach to improving CRS treatment [3].

In 2019, Norelli et al., a team well known for their prior research on CAR-T effector cell toxicities, demonstrated the crucial role of IL-6 in CRS onset with the use of a preclinical mouse model [8]. They found that high levels of IL-6 closely correlated with the severity of systemic inflammation, where hyperpyrexia, vascular leakage, and multi-organ failure were the common symptoms [8]. By inhibiting IL-6 action, they achieved a decrease in serum IL-6 level of 70% or more, and the CRS symptoms became significantly better, as illustrated in Figure 1-a [8]. This solid evidence approach supported the hypothesis of IL-6 as a central driver of CRS and that the blocking of IL-6 is a possible anti-CRS strategy [8].

This same year, Liu et al., who are hematologists working on CRS in patients with hematologic malignancies, reported the results of a retrospective analysis of CRS patients [11]. They found that patients with severe CRS showed significantly higher IL-6 levels than patients with mild CRS [11]. Further, their data indicated a positive correlation of the level of IL-6 with the degree of clinical severity, illustrated in Figure 1-b, including higher temperature and higher degree of organ dysfunction. Particularly, patients with severe CRS had serum IL-6 levels of 3054.55 ± 3698.03 pg/mL, in comparison to 286.67 ± 896.34 pg/mL in CRS the severity was mild and the correlation of

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the two variables was statistically significant [11]. In addition to making clinical discoveries that correlate with preclinical evidence, they confirmed the hypothesis that IL-6 is the most relevant therapeutic target in combating CRS [11]. Collectively, these research results showed the importance of formulating precise IL-6-directed techniques for improving CRS treatment in CAR-T cell therapy [11].

Building on the previous research regarding the central role of IL-6 in CRS, Sterner et al. (recognized pioneers in determining the interrelation of GM-CSF and CRS pathophysiology) took a deep approach using a murine xenogeneic model in order to reveal the cascade events related between GM-CSF and IL-6 in the progression of CRS [12]. Their results revealed that GM-CSF blockers can be used effectively with rare toxicities and reduce neurotoxicity symptoms, such as encephalopathy [2, 12]. However, systemic effects of CRS, for example, fever, hypotension, and inflammation, were not resolved due to high levels of serum IL-6 [12]. Even with GM-CSF inhibition, the serum level of IL-6 was kept at a high value, so the involvement of IL-6 as a major mediator for systemic inflammatory responses in CRS was evident [12]. This work not only underscored the fact that GM-CSF inhibition is unable to completely stop the CRS but also emphasized the significance of directly targeting IL-6 to control full-blown systemic CRS symptoms [12]. Furthermore, their studies gave unprecedented evidence that the passage of the GM-CSF inhibition technique would not be a viable option for full-fledged CRS management, thus assuring that the focus of the research should remain on IL-6 [12].

In 2022, Zhang et al. (a group specializing in cytokine signaling and CAR-T therapies) published a pioneering work of CAR-T cell genetic modification using short hairpin RNA (shRNA) to simultaneously knock down IL-6 and interferon-gamma (IFN-γ) [4]. This dual-targeting strategy aimed to mitigate CRS by targeting two or more inflammatory pathways [4]. Their experiments showed that the reduction in the levels of IL-6 mRNA was about 60% and the reduction in the levels of IL-6 protein 50%, while the levels of IFN-γ mRNA and protein levels decreased by 55% and 40%, respectively, as illustrated in Fig. 1-c [4]. These findings thus pointed to the efficacy of targeting these cytokines in suppressing inflammatory responses [4]. Notably, the investigated with IL-6R high knockdowns(s) together with TNF-α, IL-2, and IL-10 cytokines downwards by 30, 40, and 35%, respectively, which might indicate that the dual knockdown(s) substantially reduced the inflammatory cascade(s) responsible for CRS in MDS [4].

Importantly, the CAR-T efficacy is again unaffected by the above genetic modifications. At an effector-to-target (ET) ratio of 3:1, the CAR-T cells genetically modified have a killing potency that preserves more than 95% of themselves compared with not modified CAR-T cells, as is shown in Figure 1 -b [4]. This method preserved the therapeutic potency and simultaneous suppression of inflammation which has shown the potential of these mechanisms of CRS addressed by this intervention [4]. It is undoubtedly that adding IL-6 knockdown therapy into the plan of action for CRS treatment will be the best clinical option without compromising CAR-T cell potency.

Demonstration of clinically relevant IL-6 knockout and its functional aspects underscores the importance of fully translating bench research into CAR-T therapy [1]. More and more evidence strongly supports the feasibility and potential advantages of targeting IL-6R in managing CRS [4, 8 12]. Collectively, while pharmacological inhibitors permitted partial relief just only by disrupting the IL-6 signaling, they were unable to encompass the whole prevention of IL-6 overproduction and also its systemic effects. In contrast, genetic strategies, such as using CRISPR-Cas9 to knock out the IL-6R on CAR-T cells, are better suited [6]. By directly inhibiting the activation of IL-6-mediated inflammatory pathways, these genetic modifications could provide a more robust and preventive solution for CRS management, improving both the safety and efficacy of CAR-T therapy [8, 12].

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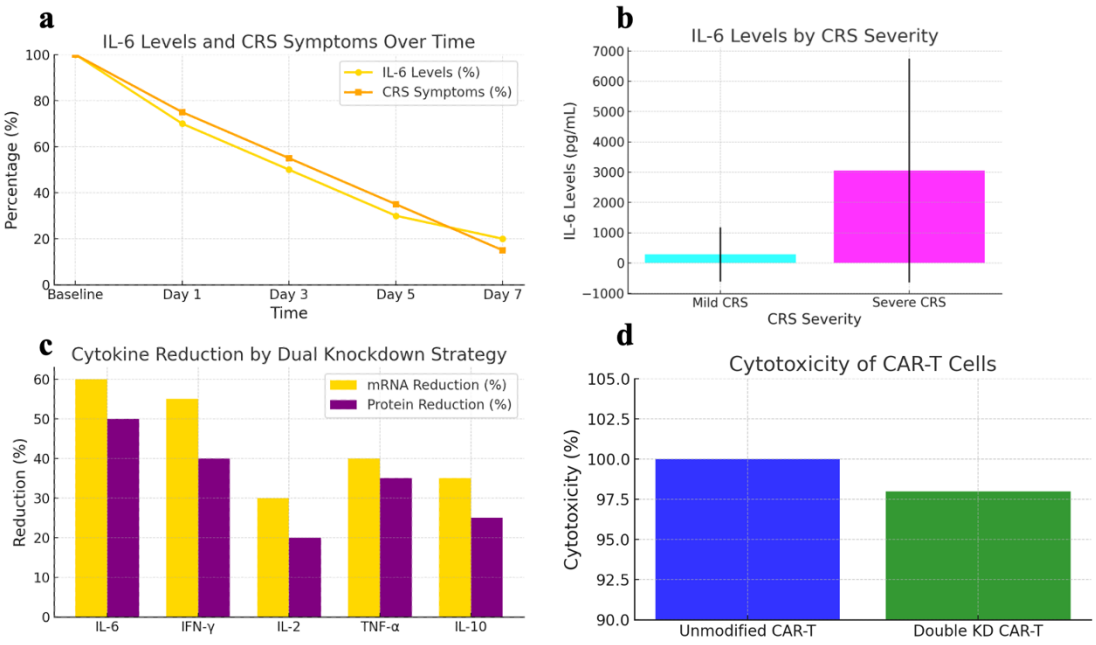

Figure 1: The central role of IL-6 in CRS and the significance of its targeted inhibition in reducing inflammation, improving symptoms, and maintaining CAR-T efficacy [4,8,11,12].

Figure 1 collectively illustrate the critical role of IL-6 in CRS pathogenesis and the effectiveness of targeting IL-6 and IFN-γ to mitigate CRS symptoms while preserving CAR-T cell cytotoxicity [4, 8, 11, 12]. The correct data underscore that blocking IL-6 signaling gives significant decreases in IL-6 concentration, symptoms associated with CRS resolution, and broad inflammation suppression, particularly in severe CRS cases. It is noteworthy that the cytotoxic ability of CAR-T cells remains intact even after genetic alterations.

4. Challenges and Future Strategies for IL-6R Knockout in CRS Management

To expedite CRS amelioration, this review proposes knocking out IL-6R using the CRISPR-Cas9 technology platform, which is now known to be a perfect gene-editing tool, yet safety and efficacy concerns are prevailing [8, 13]. Off-target effects are one of the major problems due to unintended edits at non-specific genomic sites that may impair essential cellular functions or trigger adverse events, including tumorigenesis and immune dysregulation [6]. To solve this problem, this will be followed by the development of high-fidelity Cas9 variants like SpCas9-HF1 and eSpCas9 that provide higher editing accuracies and the best designs or computational tools used for the guide RNA (gRNA) to improve the CRISPR applications feasibility [10]. Additionally, Transient delivery systems, including mRNA or ribonucleoprotein (RNP) complexes, also help to improve the safety for gene-editing of components by limiting the duration to avoid permanent off-target mutations [14].

Another significant challenge lies in ensuring a constant level of efficiency and effectiveness in gene editing in CAR-T cells. The methods for the delivery of electroporation and viral vectors often do not only enhance the risk of cell viability loss but also generate inconsistent editing efficiencies [14]. The most useful advances have been shown in optimized electroporation techniques and lipid nanoparticle systems [14]. They maintain action cell integrity while achieving uniform editing [14]. It will also be a great step to standardize these technologies because it could enable large-scale CAR-T production [1] Additionally, further preclinical validation and extended follow-up for the persistence of genetically modified CAR-T cells and long-term tumor-targeting ability is a challenge also needed to ensure [7].

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Continuing along this line of thought, integrating IL-6R knockout with other genetic alterations offers a core opportunity for extending the therapeutic use of CAR-T therapy [4]. Matching IL-6R knockout with strategies to enhance CAR-T cell persistence as well as resistance to immunosuppression in neoplasms, would enhance the efficiency and effectiveness of therapies [1]. On the other hand, CRISPR could also be further optimized to minimize off-target effects and enhance the precision of the clinical-grade CAR-T cell manufacturing process due to base editing and prime editing [10]. A result of these innovations is that they create a new safety standard as well as make it possible to reduce regulatory difficulties that give rise to next-generation CAR-T therapies that are both faster and safer and can be developed for a broader range of types of cancer [1, 4].

5. Clinical Implications of IL-6R Knockout in CAR-T Therapy

The strategy of knocking out IL-6R in CAR-T cells could potentially turn out to be a very fundamental step for the safety and effectiveness of CAR-T cell therapy to the maximum level [3]. This method directly targets the excessive IL-6 signaling responsible for CRS, not only reducing the critical life-threatening issues but also preserving the tumor-killing functions of these CAR-T cells [4]. With an improved safety profile of IL-6R knockout, CAR-T therapy can be broadened to patients who are with advanced malignancies or with pre-existing medical conditions [1]. In the same way, lessening systemic inflammation risk may help patients' tolerance for higher doses of CAR-T cells which leads to overall intensification of therapeutic efficacy and high effectiveness [5].

Moreover, IL-6R knockout entails an important opportunity to make CAR-T therapy possible on solid tumors [1]. By expediting the integration of CAR-T therapy with systems that involve immune checkpoint inhibitors as alternatives to CAR-T therapy that would require these treatments to be processed first, more comprehensive and precise cancer therapy solutions will be possible [15]. Importantly, these IL-6R knockout variants represent a major step towards understanding not only the potential but also the impact of CAR-T cancer therapy in the oncology spectrum.

6. Conclusion

CRISPR-Cas9-mediated IL-6R knockout represents a transformative approach to addressing CRS, which is one of the most significant challenges associated with CAR-T cell therapy. The direct targeting of this signaling pathway not only diminishes systemic inflammation but also maintains the killing efficacy of CAR-T cells. Recent experimental evidence is, therefore, in favor of IL-6R knockout, demonstrating substantial decreases in cytokine release while the maintaining functionality of CAR-T cells. Furthermore, the improvement in delivery systems and gene-editing precision solve the technical and safety of the CRISPR-Cas9 technology for its clinical application.

This review stresses the necessity of incorporating some relevant genetic modifications like that of IL-6R knockout among CAR-T cell engineering to boost stronger immunotherapies. The future direction of research should concentrate on the optimization of delivery systems, examination of safety and efficacy over the long term, and combining IL-6R knockout with these complementary strategies so that the CAR-T therapy would gain wider recognition in the oncology field, including solid tumor cases. It will revolutionize CAR-T therapy and have a more robust and scalable solution to manage its associated toxicities while offering better outcomes for patients.

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