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On-target off-tumor (OTOT) toxicity from HER2-targeting chimeric antigen receptor (CAR) engineered T cell therapy: current solutions

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Abstract. Breast cancer is a major threat to women's life and health, with HER2+ BC accounting for a significant proportion of cases. This subtype is characterized by aggressive behavior, a high recurrence rate, and generally poor prognosis. The efficacy of conventional HER2-CAR-T treatment for HER2-positive breast cancer is well-documented; however, it is not without its risks, particularly the potential for on-target off-tumor (OTOT) toxicity, which poses a significant threat to patient safety. This analysis examines the difficulties encountered with standard HER2-CAR-T approaches and discusses contemporary methods designed to reduce the incidence of OTOT toxicity. This review organizes the strategies into three primary methods, offering an exhaustive summary to facilitate the medical and research sectors in gaining a deeper comprehension of the present scenario and prospective advancements of HER2-CAR-T treatment. By discussing these approaches and the underlying mechanisms that make them effective, this review aims to inspire further innovation in improving existing HER2-CAR-T therapy. A thorough understanding of the current challenges and promising avenues for enhancement in HER2-CAR-T therapy is quite important for advancing future research and clinical applications.

Keywords: HER2, CAR-T cells, On-target off-tumor toxicity, Multi-antigen targeting, SynNotch.

1. Introduction

Breast cancer, which is the leading form of cancer among women, is prevalent in 157 out of 185 surveyed countries, resulting in an estimated 670,000 fatalities worldwide in 2022 [1]. Within the spectrum of breast cancer subtypes, human epidermal growth factor receptor 2-positive breast cancer (HER2+ BC) stands out, constituting approximately 20% of total breast cancer incidences. HER2+ BC is distinguished by its notably aggressive nature, elevated likelihood of relapse, and typically unfavorable outcome. These attributes collectively present substantial obstacles in the realms of efficient disease management and therapeutic intervention [2].

Over the past few years, the advent of CAR-T therapy has marked a significant advancement in medical treatment, particularly in the realm of oncology. Initially, it has been exceptionally effective in combating blood-related cancers, including leukemia. Moreover, this novel therapeutic strategy is now being explored for its efficacy against specific types of solid tumors, as indicated by recent research [3]. In 2017, the FDA granted approval for the inaugural CAR-T treatment, signifying a significant

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milestone in the field of immunotherapy. Since then, both clinical and preclinical investigations have consistently demonstrated the safety and effectiveness of CAR-T therapies that are specifically designed to target HER2 in the treatment of HER2+ BC [4, 5].

Despite these advancements, a critical challenge persists. Despite the high expression of HER2 in HER2+ BC cells, its presence is also observed on numerous normal cells. Such extensive distribution can result in OTOT toxicity, which poses challenges for the advancement and utilization of HER2-CAR-T treatment approaches. This unforeseen consequence significantly impedes the progress and utilization of HER2-CAR-T treatment. Addressing this issue is crucial for minimizing adverse effects and improving patient outcomes. Consequently, reducing OTOT toxicity has become a major focus of current research in the field.

This scholarly article seeks to deliver an exhaustive examination of the diverse tactics that have been suggested to reduce the toxicity of OTOT effects linked to CAR-T therapy targeting HER2. Additionally, it will explore the constraints inherent in these strategies, providing valuable perspectives for upcoming studies focused on refining HER2-CAR-T treatments. The goal is to maximize the therapeutic efficacy of this approach in managing HER2+ BC.

2. Introduction of synthetic biology components

The development of the SynNotch technology marks a notable progress in the engineering of artificial receptors for T-cell treatment. This innovative approach mimics the signaling function of the natural Notch receptor but is engineered with tailored specificity to enhance therapeutic precision. Unlike traditional CAR-T cell therapies that directly engage with antigens present on both tumor and normal cells, SynNotch offers a more selective mechanism. The SynNotch receptor is engineered to bind to HER2 with a low affinity, activating only when HER2 density is high enough to trigger downstream signals. The design leads to the manifestation of a CAR with high affinity for HER2, creating a feedback mechanism that promotes the proliferation of T cells, predominantly when high levels of antigen are present. This approach reduces the potential for OTOT toxicity, as it ensures that T cells are less prone to target tissues with lower HER2 expression [6].

Recent research has shown that SynNotch technology can enhance the precision of T-cell treatments. Specifically, Axel Hyrenius-Wittsten and colleagues discovered a tumor-specific antigen known as Alkaline Phosphatase Placental-like 2 (ALPPL2). They then integrated this antigen with HER2-targeting SynNotch CAR circuits to improve therapeutic specificity. Their study encompassed both in vitro and in vivo testing, demonstrating improved tumor targeting efficacy. This underscored the promising potential of SynNotch technology to enhance the precision of T-cell responses against solid tumors [6]. In a comparable study, Rogelio A. Hernandez-Lopez and colleagues engineered a T-cell receptor circuit that exhibits an exceptionally sensitive response to antigen concentration. By incorporating a dual-stage positive feedback loop, this innovative system empowers T cells to discern between healthy cells featuring minimal HER2 levels and cancerous cells that exhibit markedly higher HER2 expression. By adjusting the affinity of the synthetic Notch receptor and the CAR, this advanced design achieves precise antigen density discrimination [7]. Their findings, which were validated through in vitro experiments, three-dimensional (3D) spheroid cell culture models, and mouse tumor models, underscore the potential of this approach to reduce off-tumor toxicity while enhancing therapeutic efficacy.

In conclusion, the integration of synthetic biology elements like SynNotch into CAR-T therapy offers promising strategies to address the limitations of traditional HER2-targeted therapies. By incorporating advanced mechanisms for antigen density perception and regulatory control, these novel approaches are paving the way for safer and more effective treatments for HER2+ BC, potentially overcoming the challenges associated with OTOT toxicity.

3. Modification of the CAR domain

Modifying the dimensions and arrangement of the extracellular spacer, hinge, and transmembrane segments of CARs represents an innovative strategy to fine-tune the responsiveness and specificity of

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CAR-T cells in relation to antigens. Recent research indicates that shortening these regions could potentially boost the ability of CAR-T cells to detect cells with a high concentration of antigens, concurrently diminishing their sensitivity to cells with a lower antigen concentration.

For example, altering the length of the extracellular spacer can influence the spatial constraints between CAR and cell membrane, thereby diminishing the CAR molecule's mobility. This decrease in mobility enhances the specificity of CAR-T cells in identifying cancer cells that display higher concentrations of antigens. A study by Kelly T. Kennewick and colleagues, utilizing flow cytometry and animal experimental models, has shown that the use of reduced-length spacers can enhance the specificity of CAR-T cells in targeting tumor cells, especially those with elevated antigen concentrations. Furthermore, it was noted that certain spacer segments, like the HL spacer, are capable of boosting the effectiveness of CAR-T cells against cancer while reducing the impact on cells with low antigen density [8].

The hinge segment, acting as a bridge between the antibody's single-chain variable fragment (scFv) and the transmembrane domain, plays a pivotal role in the CAR's architecture. Variations in the hinge's length and composition can significantly alter the CAR's interaction with antigens present on the surface of tumor cells, which in turn can modulate the activation and signaling pathways of T cells. In a study by Scott McComb et al., adjusting the hinge length reprogrammed the antigen sensitivity of an EGFR-based nanobody CAR (EGFR-SDCAR), offering a novel optimization strategy for HER2-CAR-T therapy. The results of their study, confirmed by conducting both in vivo and in vitro tests, indicated that the modification of hinge region in CAR-T cells by shortening it can decrease their antigen reactivity, which in turn enhances their specificity in targeting tumors that have an elevated expression of EGFR [9].

In summary, modifications to the extracellular spacer, hinge, and transmembrane regions of CAR provide a promising research direction to address the OTOT toxicity associated with traditional HER2-CAR-T therapy. Researchers can enhance the efficacy and safety of treatments for HER2+ BC by adjusting the antigen sensitivity of HER2-CAR-T cells through modifications of specific domains.

4. Utilization of multi-antigen targeting

In the conventional approach of CAR-T treatment, T cells are guided to eliminate particular cancer cells by focusing on a single antigen, a strategy that frequently leads to OTOT toxicity. A new strategy that has gained traction involves simultaneously targeting several antigens present on tumor cells, aiming to improve the precision and reduce the risk associated with CAR-T therapy. According to current research, the additional antigen used in conjunction with the primary T-cell target must exhibit "specificity" within body tissues—meaning this antigen should be present exclusively in tumor cells or normal tissue cells, but not in both.

For instance, ALPPL2 is found in a range of solid malignancies, yet its presence is minimal in healthy tissues. This characteristic renders it a suitable target for CAR-T therapy, particularly when synergized with additional markers. CAR-T cells equipped with SynNotch technology have demonstrated potential in reducing the OTOT adverse effects often linked to standard HER2-directed CAR-T treatments, by enabling the recognition of both ALPPL2 and other cancer-specific antigens. In research by Axel Hyrenius-Wittsten and colleagues, it was shown through both in vivo and in vitro tests that SynNotch CAR-T cells have the capacity to target ALPPL2 alongside additional tumor-associated antigens, including MCAM, mesothelin, and HER2. This approach enhances the precision and effectiveness of the therapy [6].

Another study by David Bassan et al. identified an inhibitory domain (iDomain) named LIR1, which effectively regulates the cytotoxicity of second-generation HER2-CAR-T cells. In this research, the researchers chose HLA-A02 as an extra target for dual targeting, aiming to enhance specificity. This target is typically found in normal cells but is notably absent in tumor cells. Their research demonstrated that utilizing a NOT gate mechanism by combining an inhibitory CAR (iCAR) targeting HLA-A02 with an activating CAR (aCAR) targeting HER2 could effectively address the OTOT toxicity seen in traditional HER2-CAR-T therapy [10].

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In conclusion, integrating a distinct secondary antigen with the principal antigen in CAR-T treatments is an emerging approach that may help to reduce the OTOT toxicity typically associated with standard methods. Further exploration in this domain could pave the way for overcoming the obstacles linked to HER2-CAR-T interventions.

5. Other solutions

To address the OTOT toxicity associated with traditional HER2-CAR-T therapy, in addition to the three general strategies mentioned previously, several innovative methods have been developed that warrant further exploration. These methods include optimizing the affinity of single-chain variable fragments (scFvs) through screening and mutation, as well as leveraging the hypoxic characteristics of most solid tumors to enhance CAR-T cell tumor recognition.

5.1. Optimization of scFv affinity

The scFvs consist of a single-chain polypeptide that includes two components from the variable region of an antibody: the VH (heavy chain variable region) and the VL (light chain variable region). Genetic engineering can be utilized to modify ScFvs, enabling the selection of those with the most suitable affinity. This optimization enhances the specificity of CAR-T cells in targeting tumor cells, thereby reducing potential harm to healthy tissues. In their exhaustive analysis, researchers led by Yanting Duan determined that CAR-T cells engineered with low-affinity scFvs are capable of minimizing harm to healthy cells that exhibit minimal antigen presence, all the while maintaining a strong capacity to destroy tumor cells. Beyond affinity, other factors such as the scFv structure, linker peptide length, choice of co-stimulatory domain, and activation domain strength significantly influence CAR-T cell performance [11].

5.2. Use of hypoxia-inducible transcription amplification (HiTA) system

Hypoxia, which is defined by reduced oxygen levels resulting from inadequate blood circulation, is a common feature observed in the majority of solid tumor types. By leveraging this characteristic, scientists have crafted techniques for regulating the expression of CARs within T cells, contingent upon distinct microenvironmental conditions, like hypoxic environments, to boost the precision of tumor targeting and minimize OTOT effects and toxicities. Huan He et al. demonstrated through flow cytometry and in vivo mouse model experiments that HiTA-CAR-T cells can enhance in vivo safety. Significantly, cells engineered with HiTA-CAR-T technology demonstrated no harmful effects on healthy liver tissues that harbor human HER2 proteins, in contrast to the pronounced toxicity observed with traditional CAR-T cells, as reported in reference [12]. Although this approach has not yet directly resolved the OTOT toxicity in traditional HER2-CAR-T cell therapy, it holds promise as a future solution.

5.3. Combination of Approaches

These methods and strategies can be combined to achieve better therapeutic outcomes. For instance, Axel Hyrenius-Wittsten et al. successfully combined ALPPL2 targeting with the HER2-targeting SynNotch CAR circuit, incorporating elements of synthetic biology to create a multi-antigen targeting system. This approach significantly improved the specificity of CAR-T cells for tumor cells [6]. Similarly, simultaneous optimization of scFv affinity, modification of CAR domains, and the introduction of synthetic biology components can be explored in future studies to enhance therapeutic efficacy and safety.

6. Conclusion

Over the past few years, breast cancer has emerged as the leading malignancy affecting women worldwide. Specifically, the HER2+ BC stands out due to its notably aggressive nature, propensity for recurrence, and typically unfavorable outcomes. The development of effective treatment strategies for HER2+ BC has thus become increasingly urgent. CAR-T therapies initially demonstrated high

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efficacy in treating HER2+ BC, but clinical practice over time has revealed several adverse reactions, including OTOT toxicity.

In order to tackle these issues, several effective strategies have been identified. A notable method involves the creation of CAR-T cells that can target multiple antigens. For instance, CAR-T cells designed to hit two targets at once, like those that aim for both HER2 and HLA-A02, or alternatively, ALPPL2 and HER2, are being explored. Another strategy involves the introduction of synthetic biological components into CAR-T cells, enabling control of CAR-T cell activation through engineered circuits, with the synthetic Notch (SynNotch) CAR circuit serving as a notable example.

Additionally, modifying the CAR domain to enhance the specificity of CAR-T cells for tumor cells represents a promising direction, with alterations to the extracellular spacer and hinge regions shown to improve tumor specificity. Other innovative approaches, such as optimizing the affinity of scFvs and employing hypoxia-inducible transcription amplification (HiTA) systems, also hold potential and warrant further investigation.

Ongoing research and innovation are vital for enhancing and perfecting these therapeutic approaches, with the aim of offering patients with HER2+ BC safer and more potent treatment options. The drive towards these advancements is paramount for optimizing patient results and for reducing the negative impacts of existing treatment methods.

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