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CAR T Cell Therapy: Evolutions and Emergent Improvements

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Abstract. One of the latest developments in the field of anti-cancer immunotherapy is chimeric antigen receptor (CAR) T cell therapy, which is considered magnificent and impressive owing to its superior response rates and efficacy. It has been proven to exhibit great efficacy in curing patients with B cell lymphoma and leukemia, and sometimes multiple myeloma as well. Nevertheless, despite the significant outcomes it has displayed, several limitations and restrictions still deeply restrain CAR T cell therapy from being widely used throughout the world. Particular challenges associated with T cells range from severe cytokine-related toxicities, the on-target off-tumor effect, and antigen escape, to inferior CAR T cell trafficking and immunosuppressive tumor microenvironment. To address the related problems, investigators learn from the evolutions of CAR design and incessantly attempt to modify and ameliorate CAR constructs and launch it to the wider extent of solid tumors and malignancies. A large array of methods and strategies have been adopted by investigators to fulfill this goal, ameliorating the efficacy, security, and applicability of CAR constructs. In this review, we will concentrate on the evolutions of CAR designs, and several limitations CAR T cell therapy recently faces, along with some innovative strategies come up by researchers to tackle them and explore the clinical benefits of this therapy against various cancers.

Keywords: CAR T cell therapy, chimeric antigen receptors, cancer immunotherapy, B cell acute lymphoblastic leukemia

1. Introduction

Cancer is always regarded as an unprecedentedly risky and hazardous disease, with extraordinarily high incidence and death rates. In the United States, 609,360 cancer-related deaths are anticipated in 2022, along with roughly 1.9 million new cancer cases [1]. However, traditional therapies for cancer, including surgery, chemotherapy, and radiation, have several limitations and pitfalls nowadays, ranging from severe pain, nerve problems, the resistance to chemical agents, to the need for combination therapy to cure the patient. In contrast, immunotherapy offers long-term cancer remission to patients, compared to traditional therapies, in order to lower the death rate and the possibility of relapse. Therefore, immunotherapy prevails around the world at present, in which Chimeric Antigen Receptor (CAR) T-cell Therapy is frequently viewed as a treatment option for a number of malignancies if the other therapies show no promising sign of recovery. Chimeric antigen receptors are essentially receptor proteins that have been genetically altered to enable T cells to target a particular antigen. These receptors are referred to as "chimeric" because they have the capacity to attach to an antigen as well as trigger an immune response in T cells. They can cause lymphocytes,

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particularly T cells, to be redirected in order to identify and attach to particular antigens produced on the intended cells, which will cause their eradication [2]. T cells that express CARs on the cell membrane are regarded as CAR T cells. For the most part, this technology is widely used to target specific cancerous cells in the human body. The Food and Drug Administration approved the use of CAR T cells targeting CD19 in 2017 as the first treatment strategy containing a genetically modified component for use across the United States. Currently, the success rate of CAR T-cell therapy for long-lasting remission without accompanying treatment ranges between 30% and 40%. Recently, researchers have focused their efforts on combining CAR T cell therapy with a number of other anticancer treatments, enhancing the structure and design of CARs to avoid potential risks and production problems associated with existing agents, and improving the clinical efficacy and capacity to overcome resistance. In the past, CAR T cells only showed relatively promising effects on patients who suffered diffuse large B cell lymphoma (DLBCL) or B-cell acute lymphoblastic leukemia (B-ALL) [3]. However, the efficacy of novel CAR engineering tactics in treating ALL and B cell lymphoma can now be extended by researchers to patients with additional malignancies as well as solid tumors. Through this review, the evolutions and structure of CAR will be first introduced, following the manufacturing and massive production of CAR products, and finally some emergent strategies that can tremendously ameliorate the overall effectiveness and safety of CAR products, with which a wide spectrum of solid tumors and hematological malignancies can be readily handled by researchers.

2. CAR evolutions

CAR T cell therapy is undoubtedly a historically long-lasting therapy, spanning from 1989 to recent days. Along its way of development, four generations of CAR have been devised and introduced by scientists. History of development of CAR effector cells. CAR stands for chimeric antigen receptor, CRISPR stands for clustered regularly interspaced short palindromic repeats, FDA stands for US Food and Drug Administration Agency, EMA stands for European Medicines Agency, DLBCL stands for diffuse large B-cell lymphoma.

At the Institute for Comprehensive Medical Science located in Aichi, Japan, Yoshihisa Kuwana et al. produced chimeric receptors by combining parts of an antibody and the T cell receptor, which were typically regarded as the initiation of CAR design. Independently, Gideon Gross and Zelig Eshhar at the Weizmann Institute in Israel also prescribed the same receptor in 1989 and termed it as ‘T body’ [4]. These early approaches, though not effective clinically speaking, integrated the constant regions of T cell receptor proteins with an antibody's capacity to attach to certain targets [4]. In 1991, Arthur Weiss, who dedicated himself to anti-cancer treatment at the University of California, San Francisco, invented the first-generation CARs [5]. Chimeric receptors with antibody-like external domains, generally single-chain fraction variable (scFv) domains, were modified by Weiss and his colleagues to include CD3 zeta intracellular signaling domains. [6]. They found that these chimeric receptors were able to activate T cell signaling, laying the foundation for the practice of these receptors for clinical use, despite the failures of several medical experiments to treat HIV or solid tumors during the mid-1990s. The first decade of the 2000s saw the introduction of second-generation CARs when CD3 zeta intracellular domains of first-generation CARs were combined with co-stimulatory domains like CD28. Superior persistence and tumor elimination were exhibited by the CARs with co-stimulatory domains in several clinical models [7]. In the early 2010s, investigators who worked at the Memorial Sloan Kettering Cancer Center, University of Pennsylvania, and NCI employed second-generation CARs to target CD19, a protein exhibited on both healthy B cells and B cells with lymphomas and leukemias. The results were quite magnificent, while plenty of heavily sick patients showed complete remissions. Owing to the great clinical efficacy, the FDA ultimately approved of the first official version of CAR products in 2017, axicabtagene ciloleucel and tisagenlecleucel, which were produced and provided under Kymriah and Yescarta, respectively [8]. Along the long road which CAR T cell therapy has gone through, it becomes increasingly effective and clinically useful. There have been six CAR T cell treatments that have received FDA approval and have all gone a long way in the medical

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context so far. Undoubtedly, there will present more types of CAR T cell therapies in the near future, which will play a more significant role in the treatment for cancers and other diseases.

3. CAR structure and design

CARs are modularly constructed receptors made up of four basic parts: an antigen recognition and binding domain, a hinge region (sometimes called a spacer), a transmembrane domain, and a signaling domain (figure 1). Each domain has a distinctive function, and the constituent protein domains can vary for achieving clinically optimal designs.

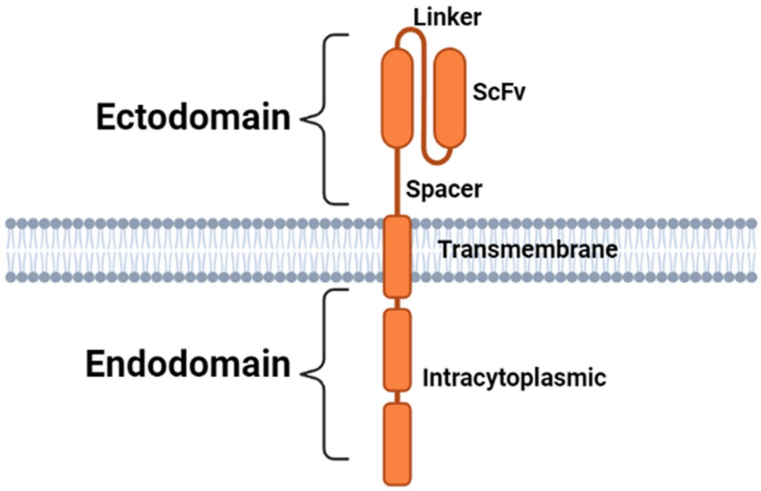

Figure 1. Structure of CARs (Created with BioRender.com). Chimeric antigen receptors, generally speaking, are composed of an ectodomain, a transmembrane domain, and an endodomain. Ectodomain is made up of a structure named ScFv, which stands for a single-chain variable fragment, and a hinge region, also known as a spacer. Endodomain consists of an intracellular signaling domain, and sometimes one or more co-stimulatory domains as well.

3.1. Antigen recognition domain

The extracellular region of CARs, known as the antigen recognition domain, confers specificity to various antigens by directing the CAR T cell to any cell carrying the appropriate antigen. These domains are often created by connecting the variable heavy (VH) and light (VL) chains of monoclonal antibodies, which are joined by a brief linker peptide. This structure is called a single-chain variable fragment (scFv). The VH and VL portions of the scFv are pre-selected to target a certain antigen.

For the most part, the surface antigens on malignant cells may be targeted by the scFvs portion in CARs, activating T cells without the assistance of a major histocompatibility complex (MHC), although sometimes MHC-dependent CARs are prescribed owing to the intracellular cancer antigens. The scFvs are quite important in the overall efficacy of CARs, as several characteristics of and variations in scFvs can remarkably impact their potency. For instance, as it substantially determined the function of CARs, the affinity between the CAR and its epitope is a crucial component to take into account. To bind to the appropriate antigen, start the cascade of cell signaling, and activate the T cell to fight the cancerous cell, the antigen binding affinity of a CAR should be at a balanced point, in which CAR T cells can be normally activated, without being killed by activation-induced death and releasing toxicities. As the research shows, the mode of interaction among light and heavy chains of scFvs can enormously influence the affinity of specificity of the CAR to its antigen's epitope [9]. In

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other words, scFvs with similar, but not exactly the same, affinity can lead to notable variation in CAR functions. Hence, on account of optimizing the effect of CAR to target specific antigens, some other parameters must also be taken into account, including antigen density, the site of the targeted epitope, the possibility of tonic (ligand-independent) signaling, and so forth.

3.2. The hinge

The area between the transmembrane domain and antigen recognition domain is known as the hinge region, also known as the spacer. It could especially offer the flexibility to get beyond the steric hindrance effect and length for the scFv head to contact particular antigens. Different lengths and chemical makeup of the hinge can remarkably influence the CAR's antigen-binding and signaling ability, while the length of the spacer is also integral to offering sufficient space for immunological synapse formation. The hinge's amino acid sequences are often based on other immune molecules, including CD8, CD28, IgG1, and IgG4, all of which allow the CAR to reach the epitope, despite the potential risk of CAR T cell elimination and decreased CAR persistence owing to the interaction with Fcγ receptors [10]. However, these risks can be hopefully avoided through either selecting another hinge region, or additional modification to the spacer region on account of its function and structure.

3.3. Transmembrane domain

The transmembrane domain is embedded in the T cell's membrane and is responsible for anchoring CARs into the membrane of T cells. According to research thus far, the transmembrane domain can interact with innate signaling molecules, be engaged during cell signaling and immunological synapse formation, and impact the expression and stability of CAR. Typically, transmembrane domains come from proteins like CD3 Zeta, CD28, CD4, and so forth. The differences between them as the transmembrane domain remain unclear, due to the requirement for external recognition domains or intracellular signaling regions and the concomitant frequent alteration of the transmembrane domain. Of note, the CD3 zeta transmembrane domain can promote CAR-mediated T cell activation by participating in CAR dimerization and being incorporated into endogenous TCRs. However, the transmembrane domains that are mainly being used are CD28 and CD8α, since CAR stability is sacrificed for appropriate CD3 zeta transmembrane domain function. The transmembrane domain as well as the hinge region, in general, can affect the production of CAR T cell cytokines and activation-induced cell death (AICD).

The quantity of TNF and IFNγ released by CAR T cells with CD8 as the transmembrane and hinge regions can be reduced, which means the decreased susceptibility to AICD compared to those with CD3 zeta and CD28, while the ones with CD28 transmembrane domains can largely enhance CAR stability [11]. In conclusion, the best possible CAR T cell signaling may be accomplished through matching the nearby intracellular signaling domain to the corresponding transmembrane domain, and by utilizing the most common CD28 and CD8α domains.

3.4. Intracellular signaling domain

The signaling domain of a CAR is presumably the most important and the one that is being studied the most, while most of the endeavor and effort upon the intracellular domain are concentrated on understanding and constructing the optimal co-stimulation domain to generate the best clinical effect. An activation domain plus one or more co-stimulatory domains often make up a CAR's intracellular signaling domain. To generate effective T cell responses, these activation motifs alone cannot signal sufficiently, which may lead to restricted in vivo durability and persistence, supported by several clinical studies, saying that the first-generation CARs with only one activation domain showed limited or even no efficacy. Hence, one or more co-stimulatory domains are always needed to support the CAR T cell's function and metabolism and bring out the ideal medical effect. A well-known study on the proliferation of IL-2 after repeated antigen exposure revealed that the addition of a co-stimulatory domain significantly increased IL-2 production [12]. By comprehending the significance to add a co-stimulatory domain to the initial CD3 zeta activation domain in order to optimize the clinical

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efficacy, the second-generation CAR occurred with a co-stimulatory domain, particularly CD28. Most recently, CD28 and CD137 (also known as 4-1BB), which are both FDA-approved and have significant response rates, are the co-stimulatory domains with the greatest range. The primary areas of difference between these two co-stimulatory domains are their functions and metabolism. While CAR T cells with 4-1BB will develop into central memory cells and demonstrate elevated mitochondrial biogenesis and oxidative metabolism, those with CD28 domains will develop into effector memory cells and predominantly exploit aerobic glycolysis [13]. The generation of CARs with more than two co-stimulatory domains along with CD3 zeta is utilized with great range recently.

4. The manufacturing and production of CAR T cells

First, the researcher will separate the leukocytes from the blood, and return the remains, such as red blood cells and platelets, back to circulation. When sufficient leukocytes are collected as the product of leukapheresis, T cells will be especially enriched. Viral vectors encoding the CAR are incubated with T cells when it comes to the activation process. The viral vector first attaches to the cells of the patient, then, once it enters the cell, the vector will incorporate its genes encoding CARs into the patient's DNA by reverse-transcription. Thus, the CAR expression will maintain as the T cells proliferate. Finally, the CAR will be translated by the patient's cells and expressed on the cell surface. When the cell proliferation procedure is complete,it can be injected back into the patient. The concentrated cells will next be chilled in a medium that is impermeable. As the medical product is released into the patient's body, the refrigerated cells will thaw and be transported to where the patient should be treated. Eventually, the CAR T cells can function and bind to antigens present on tumor cells under the in vivo environment (figure 2).

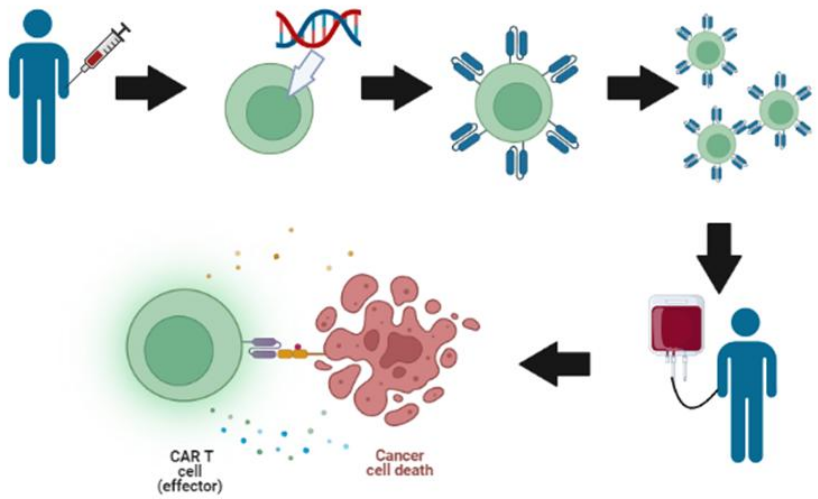

Figure 2. The simplified procedure of manufacturing CAR T cells (Created with BioRender.com).

5. Emergent improvements in CAR T cell therapy

5.1. On-target off-tumor toxicity

Since solid tumor antigens are frequently found in normal human tissues, preventing on-target off-tumor toxicity is one of the most frequent difficulties in the design of CAR T cells. Hence, getting rid of the on-target off-tumor effect also and enhancing therapeutic efficacy become two crucial factors to be counted in CAR T cells design.

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Targeting post-translational modifications that tumors have a tendency to overexpress, such as O-glycans like Tn and sialyl-Tn (STn), might be one way to prevent the risk of targeting antigens that are also expressed on normal body tissues [14]. The four key targets that are the subject of in-depth research are TAG72, B7-H3, MUC1, and MUC16 [15]. Despite the fact that the first attempt to target TAG72 in the case of colorectal cancer failed since there was no anti-tumor response, additional post-translational alterations and second-generation TAG72-targeted CAR T cells are currently the focus of much research.

There are still plenty of innovative methods that could be used to restrict and counter on-target off-tumor toxicity, one of which focuses on tapping into the hypoxia-inducible factor 1α degradation pathway [16]. Through this pathway, CAR expression can be limited only to T cells that exist in the hypoxic microenvironment, thereby restricting the potential damage and adverse effects on normal tissues, which are typically non-hypoxic. Meanwhile, using mRNA instead of DNA vectors to create CAR T cells allows for transient expression of CARs, which can help decrease the likelihood of attacking normal cells [17].

The insertion of CARs to some T cell subsets with improved safety as well as anti-tumor efficacy is one potential strategy for reducing the potential toxicity of CAR T cells. For instance, γδT cells, a subgroup of T cells that expresses T cell receptors with γδ subunits rather than common αβ subunits, are naturally able to detect tumors due to their capacity to recognize antigens that are characteristic of cancer cells with dysfunctional metabolism. An investigation examining the transduction of γδT cells expressing a GD2-targeted CAR with a co-stimulatory domain revealed that only GD2+ neuroblastoma cells were the target of cytolytic reactions, whereas GD2+ normal tissues were unharmed. The outcomes generally demonstrate how this method will probably increase safety.

One particularly bold strategy for minimizing on-target off-tumor toxicity involves genetic modifications of non-malignant body tissues to get rid of the targeted antigen. This strategy was developed as a result of the lack of CAR products for AML, which has a dearth of cell-surface antigens expressed on tumor cells that are also absent on neutrophils or essential progenitor cells. By using the CRISPR-Cas9 method to eliminate the non-essential CD33 surface marker expressed on normal progenitor cells, Kim et al. were able to get over this technical hurdle [18]. After that, when CAR T cells directed against CD33 were introduced to mice with CD33+ AML, the altered bone marrow and myeloid cells that express CD33 were not attacked. However, the effectiveness of this strategy in human bodies is yet undefined, necessitating a huge number of in vitro investigations to confirm its possible safety profile.

5.2. CAR T cell trafficking

CAR T cell therapy tackling solid tumors faces a more severe predicament compared to hematological malignancies since CAR T cells are generally constrained in their mobility and the capability of penetrating tumors by their immunosuppressive milieu and physical obstacles like the tumor stroma. CAR T cells that target IL13Ra2 and HER2 have been shown to have greater therapeutic effectiveness in preclinical studies [19]. Administration of CAR T cells in glioblastoma, leptomeningeal metastases, as well as malignant pleural mesothelioma are among the ongoing preclinical investigations [20]. Introducing chemokine receptors to CAR T cells that react to the tumor-derived chemokines is one emerging technique with superior CAR T cell trafficking. Recent research has shown that integrin αvβ6-CAR-T cells that are engineered to express or overexpress CXCR1 or CXCR2 exhibit enhanced trafficking and superior efficacy [21]. Physical obstacles like the tumor stroma will put limitations on CAR products as well by preventing tumor penetration. However, some approaches have come up to resolve this predicament. For instance, tumor stroma mainly consists of extracellular matrix which is mostly composed of heparin sulfate proteoglycan (HSPG), meaning that T cells have to degrade it so as to get access to the tumor [22].

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5.3. Antigen escape

Most often, antigen escape is one of the most severe and common problems faced by CAR-related treatment. In spite of promising therapeutical outcomes induced by CAR T cells at the very beginning, Antigen escape, also known as partial or even full loss of antigen expression, is a characteristic of the cancerous cells in a striking fraction of patients. For instance, according to clinical reports and institutional studies, some 7–25% of patients who receive CD19-targeted CAR T cell treatment will experience a CD19- disease recurrence [23]. Similar to this, after undergoing BCM-targeted CAR T cell therapy for multiple myeloma, patients have been seen to lose BCMA expression [24]. Comparable antigen escape mechanisms have also been shown in solid tumors, as tumor recurrences in glioblastoma patients who received IL13Ra2-targeted CAR T cell treatment will show reduced IL13Ra2 expression.

Scientists have focused on targeting several antigens to decrease the likelihood of antigen escape and the recurrence rate of CAR T cell therapies both for solid tumors and severe malignancies. At present, there are two strategies largely being used: one is dual CAR construct, which means using distinctive CAR T cell designs to target some optional antigens, and the other is tandem CAR, which means using CARs containing two scFv domains to target multiple tumor antigens. On the one hand, dual CARs can be produced by blending a range of CAR T cells aimed at single antigens, or by the transduction of T cells with multiple CAR constructs. On the other hand, tandem CAR can be created by adding two binding domains to a single CAR molecule. So far, it appears that both of these strategies have demonstrated great clinical efficacy, and several CD19/CD20 or CD19/CD22 trials go a long way. Tandem CARs have been used in multiple clinical studies for solid tumors, such as those for MUC1 and HER2 in breast cancer and IL13Ra2 and HER2 in the case of glioblastoma, both of which exhibit greater therapeutic performance than single target treatment [25].

Further CAR product alterations are required to make them express bi-specific T cell engagers (BiTEs), which is another emerging multi-antigen treatment. Typically, BiTEs are composed of two scFvs, each of which is particular to CD3 or a tumor-associated antigen, respectively. They are connected by a linker, which endows BiTEs with the ability to physically connect T cells with cancer cells. Of note, blinatumomab, a therapy based on BiTEs CAR T cells, has been approved by FDA against ALL in recent years. Researchers have already and still continue to dig into the potential of BiTEs to minimize the probability of antigen escape for both malignancies and solid tumors.

To tackle multiple TAAs, one remarkable strategy is to develop CAR T cells that can trigger a series of autologous immune responses apart from CAR T cells themselves. These products, known as armored CAR T cells, are combined with immunomodulatory substances that can control other immune system cells in the patient's body. As an illustration, CAR T cells that have been precisely designed to secrete the proinflammatory molecule CD40 ligand (CD40L) can serve as an illustration. Because CD40L co-stimulation gives CAR T cells better intrinsic functioning, they may stimulate antigen-presenting cells and significantly boost tumor cells' immunogenicity, which encourages autologous T cells to recognize and kill tumor cells without the help of CAR T cells [26].

5.4. CAR T cell associated toxicities

According to reports from patients with lymphoblastic leukemia or lymphoma (ALL/LBL) receiving CAR T cell treatment, almost all patients have experienced toxicities of some severity, and 23–46% of patients have experienced massive T cell expansion or cytokine production [27]. The cytokine-released syndrome (CRS), which would be characterized by severe cytokine production and intensive T cell expansion [28], and macrophage activation syndrome (MAS).

Other than CRS and MAS, there are still several severe toxicities potentially bred by CAR and its related products. A neurological disorder known as immune effector cell-associated neurotoxicity syndrome (ICANS) is linked to a disturbed blood-brain barrier and an elevated amount of cytokines in cerebrospinal fluid (CSF). The common symptoms include tremors, seizures, headaches, and hazardous cerebral edema, sometimes accompanied by CRS.

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Pathologically speaking, CRS can be mediated by IL-6, which is heavily relied on to combat this toxicity. In the meantime, increased comprehension and appreciation of these toxicities lead to some improvements in clinical management, including the use of IL-6. To reduce the possible negative impact of corticosteroids on the anti-tumor function of CAR T cells, agents that target IL-6 pathways, such as tocilizumab and siltuximab, are being utilized in conjunction with or as alternatives for corticosteroids [29]. It's widely believed, though paradoxical, that the potential of cytokine-related toxicities can diminish by infusing fewer CAR T cells. However, this association is slightly inaccurate [30], therefore the best way to minimize toxicities is to fundamentally modify CAR constructs.

The choice of co-stimulatory domains on the intracellular signaling domain is quite important in dictating related toxicities. For example. The initiation of T cell responses and subsequent depletion are considered to occur more quickly with CD28 co-stimulatory domains than with 4-1BB domains. In other words, 4-1BB domains are regarded to have a less intense T cell proliferation, resulting in increased CAR T cell durability and a decreased danger of toxicities brought on by cytokines. Hence, the decision of co-stimulatory domains based on tumor burden, the targeted antigen, the specific scFv, and other factors provide the CAR design with a readily-controlled variable. In the case of choosing between 4-1BB and CD28, 4-1BB would be superior if the antigen density or tumor burden of the patient is relatively high, and will result in less toxicity and better efficacy, while CD28 would be more proper to use if the antigen density is low or if the binding affinity of the scFv is poor [31].

Besides intracellular signaling domains, modifications of certain other domains can lower the risk of cytokine-related toxicities. For instance, modifications based on the fundamental amino acid sequences of the transmembrane and spacer domain, which are obtained from CD8α, of a CD19-directed CAR construct appear to induce lower levels of T cell proliferation and cytokine release [32]. In the same phase 1 trial, Ying, Z. et al. found that these CAR constructs resulted in complete remissions in approximately 54.5% of patients who suffered from B cell lymphoma.

Other than modifications to CAR design, the recognition and reaction of the host immune system may be also associated with cytokine-related toxicities. Thus, the use of antibodies derived from the in vivo environment instead of from mouse bodies, accompanied by some modifications of the hinge or transmembrane region will remarkably alleviate the immunogenicity of CARs [33]. More significantly, this approach appears to increase CAR T cell durability and reduce the likelihood of toxicities associated with cytokines [33].

5.5. Immunosuppressive microenvironment

The tumor microenvironment largely dictates the effectiveness and durability of CAR-related treatment. Numerous cell types, including myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and tumor-associated macrophages (TAMs), can augment tumor infiltration in the tumor microenvironment [34], which will considerably promote the production of tumor-facilitated chemokines, growth factors, and cytokines. In addition, immune checkpoints like PD-1 or CTLA-4 also exert influence on anti-tumor ability. Notably, inferior T cell proliferation and brief CAR T cell durability are two of the primary reasons for a weak or even nonexistent response to CAR products. Co-stimulatory pathways are also widely thought to be the cause of T cell exhaustion and depletion. [35]. Hence, the combination immunotherapy of CAR-related therapies with immune checkpoint inhibitors would be the first-line field to be investigated. The advantages of the combination therapy are conspicuous, for CAR T cells will recognize and eliminate cancerous cells and immune checkpoint blockade will confer sustained T cell function [36]. In a study held at Children's Hospital of Pennsylvania aimed at 14 children with serious B-cell acute lymphoblastic leukemia, also widely known as B-ALL, children who were provided with CD19 CAR T cell treatment in combination with PD-1 checkpoint inhibitor displayed better outcomes.

Modifying CAR T cells to release immunostimulatory signals that take the form of stimulatory cytokines is another cutting-edge research of changing the milieu to promote CAR T cell effectiveness. This can increase the proliferation and survival of CAR T cells and balance the tumor environment. Plenty of investigations and studies have focused on the expression of several pro-inflammatory

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cytokines, such as IL-12 and IL-15, instead of inhibitory signaling, and then redirecting the immunosuppressive cytokines, like IL-4, towards proinflammatory cytokines.

Despite the successful precedents above, it should be recognized that combination therapy would only provide a brand-new option for immunotherapy, it might still fall short and be inefficient in promoting T cell effectiveness and expansion. To better deal with the suppressive signal existing in the tumor environment, more combination immunotherapy needs to be tested and studied in the context of complicated hematological malignancies or tumors.

6. Conclusion

As the pioneer in therapies that entail genetic modification, CAR T cells are magnificent immunotherapy based on the engineering of T cells, offering us spectacular opportunities to explore the field of cancer biology. Hopefully, it will enter the mainstay of treatments for B cell malignancies and several other cancers within a few years. The toxicities associated with it have continued to cause serious concern for researchers, therefore there are still certain barriers preventing the ultimate use of CAR T cell therapy in the clinical setting. As the result, several approaches are under progress right now to remarkably improve the efficacy and efficient manufacturing of CAR products, while various trials are currently being conducted in order to broaden the applicability of CAR T cells. We hope to see the benefits and potency of CAR T cell therapy being maximized in the near future.

References

[1] Cancer Facts & Figures 2022. https://www.cancer.org (2022)

[2] Jackson, H. J., Rafiq, S. & Brentjens, R. J. Driving CAR T-cells forward. *Nat. Rev. Clin. Oncol.* **13**, 370–383 (2016).

[3] 3Park, J. H. et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N. Engl. J. Med.* **378**, 449–459 (2018).

[4] 4Eshhar, Z.; Bach, N.; Fitzer-Attas, C. J.; Gross, G.; Lustgarten, J.; Waks, T.; Schindler, D. G. (1996). "The T-body approach: potential for cancer immunotherapy". *Springer Seminars in Immunopathology*. **18 (2)**: 199–209.

[5] Irving, B. A.; Weiss, A. (1991-03-08). "The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways". *Cell*. **64 (5)**: 891–901.

[6] Hege, K. M.; Roberts, M. R. (1996). "T-cell gene therapy". *Current Opinion in Biotechnology.* **7 (6):** 629–634.

[7] Sadelain, Michel; Rivière, Isabelle; Brentjens, Renier (2003). "Targeting tumours with genetically enhanced T lymphocytes". *Nature Reviews. Cancer*. **3 (1)**: 35–45.

[8] Braendstrup, Peter; Levine, Bruce L.; Ruella, Marco (2020). "The long road to the first FDA-approved gene therapy: chimeric antigen receptor T cells targeting CD19". *Cytotherapy*. **22 (2):** 57–69.

[9] Chailyan, A., Marcatili, P. & Tramontano, A. The association of heavy and light chain variable domains in antibodies: implications for antigen specificity. *FEBS J.* **278**, 2858–2866 (2011).

[10] Guest, R. D. et al. The role of extracellular spacer regions in the optimal design of chimeric immune receptors: evaluation of four different scFvs and antigens. *J. Immunother.* **28**, 203–211 (2005).

[11] Alabanza, L. et al. Function of novel anti-CD19 chimeric antigen receptors with human variable regions is affected by hinge and transmembrane domains. *Mol. Ther.* **25**, 2452–2465 (2017).

[12] Maher, J., Brentjens, R. J., Gunset, G., Rivière, I. & Sadelain, M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ /CD28 receptor. *Nat. Biotechnol.* **20**, 70–75 (2002).

[13] Kawalekar, O. U. et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T Cells. *Immunity.* **44**, 380–390 (2016).

[14] Steentoft, C. et al. Glycan-directed CAR-T cells. *Glycobiology*. **28,** 656–669 (2018).

DOI: 10.54254/2753-8818/3/20220448

[15] Hege, K. M. et al. Safety, tumor trafficking and immunogenicity of chimeric antigen receptor (CAR)-T cells specific for TAG-72 in colorectal cancer. J. Immunother. *Cancer*. **5**, 22 (2017).
[16] Juillerat, A. et al. An oxygen sensitive self-decision making engineered CAR T-cell. *Sci. Rep*. **7**, 39833 (2017).
[17] Tchou, J. et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. *Cancer Immunol. Res*. **5**, 1152–1161 (2017).
[18] Kim, M. Y. et al. Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. *Cell* **173**, 1439–1453.e19 (2018).
[19] Priceman, S. J. et al. Regional delivery of chimeric antigen receptor-engineered T cells effectively targets HER2+ breast cancer metastasis to the brain. *Clin. Cancer Res*. **24**, 95–105 (2018).
[20] Adusumilli, P. S. et al. Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. *Sci. Transl. Med.* **6,** 261ra151 (2014).
[21] Whilding, LM. et al. CAR T-cells targeting the integrin αvβ6 and co-expressing the chemokine receptor cxcr2 demonstrate enhanced homing and efficacy against several solid malignancies. *Cancers (Basel)* **11**, 674 (2019).
[22] Zhang, B.-L. et al. Hurdles of CAR-T cell-based cancer immunotherapy directed against solid tumors. *Sci. China Life Sci.* **59**, 340–348 (2016).
[23] Majzner, R. G. & Mackall, C. L. Tumor antigen escape from CAR T-cell therapy. *Cancer Discov.* **8,** 1219–1226 (2018).
[24] Green, D. J. et al. Fully human bcma targeted chimeric antigen receptor T cells administered in a defined composition demonstrate potency at low doses in advanced stage high risk multiple myeloma. *Blood*. **132,** 1011–1011 (2018).
[25] Hege, K. M. et al. Safety, tumor trafficking and immunogenicity of chimeric antigen receptor (CAR)-T cells specific for TAG-72 in colorectal cancer. J. Immunother. *Cancer*. **5**, 22 (2017).
[26] Curran, K. J. et al. Enhancing antitumor efficacy of chimeric antigen receptor T cells through constitutive CD40L expression. *Mol. Ther*. **23**, 769–778 (2015).
[27] Frey, N. V. & Porter, D. L. Cytokine release syndrome with novel therapeutics for acute lymphoblastic leukemia. Hematol. *Am. Soc. Hematol. Educ. Program*. 2016, 567–572 (2016).
[28] Sadelain, M., Brentjens, R. & Rivière, I. The basic principles of chimeric antigen receptor design. *Cancer Discov.* **3**, 388–398 (2013).
[29] Brentjens, R. J. et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci. Transl. Med.* **5,** 177ra138 (2013).
[30] Santomasso, B. D. et al. Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute lymphoblastic leukemia. *Cancer Discov*. **8**, 958–971 (2018).
[31] Salter, A. I. et al. Phosphoproteomic analysis of chimeric antigen receptor signaling reveals kinetic and quantitative differences that affect cell function. *Sci. Signal.* **11**, eaat6753 (2018).
[32] Ying, Z. et al. A safe and potent anti-CD19 CAR T cell therapy. *Nat. Med.* **25**, 947–953 (2019).
[33] Sommermeyer, D. et al. Fully human CD19-specific chimeric antigen receptors for T-cell therapy. *Leukemia* **31**, 2191-2199 (2017).
[34] Quail, D. F. & Joyce, J. A. Microenvironmental regulation of tumor progression and metastasis. *Nat. Med*. **19**, 1423–1437 (2013).
[35] Yin, Y. et al. Checkpoint blockade reverses anergy in IL-13Rα2 Humanized scFv-Based CAR T cells to treat murine and canine gliomas. *Mol. Ther. Oncolytics.* **11**, 20–38 (2018).
[36] Grosser, R., Cherkassky, L., Chintala, N. & Adusumilli, P. S. Combination immunotherapy with

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CAR T cells and checkpoint blockade for the treatment of solid tumors. *Cancer Cell.* **36**, 471–482 (2019).