CAR-T Cell Therapy: The Limitation and Solution

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Abstract. Driven by breakthroughs in biomedical research, chimeric antigen receptor (CAR) T cell therapy has been a potential new class of the therapy that is now being explored in the field of biology with regard to cancer treatment.These genetically engineered immune cells are designed to selectively target antigens expressed on tumour cells, presenting a novel avenue in immunotherapy. Particularly in liquid tumours such as acute lymphoblastic leukaemia (ALL), the noteworthy therapeutic performance of CAR-T cells highlights their promise in the field of immune treatment research.Nevertheless, despite remarkable achievements, considerable challenges persist. Issues such as an immunosuppressive tumour microenvironment (TME), antigen loss, and restricted CAR-T cell trafficking, especially in the solid tumours, present formidable obstacles. Researchers have proposed strategies that aimed at surmounting these challenges, including the dual-targeted CAR-T cells design and TME modification, which offer promising avenues for advancement. However, unresolved questions linger, emphasizing the ongoing need for innovative solutions.

Keywords: Immunotherapy; cancer; CAR-T; restriction; toxic.

1. Introduction

Cancer remains a paramount concern within the medical research community, while chimeric antigen receptor (CAR) T cell therapy is a considerable treatment modality of immense developmental promise and practical significance. The approval by the US Food and Drug Administration (FDA) of that kind of immune therapy signifies a milestone in cancer treatment, providing solutions for various malignancies, including relapsed/refractory B-cell leukaemia, multiple myeloma and lymphoma [1].

CARs represent engineered proteins that augment the targeting capabilities of immune cells. Positioned within the realm of adaptive T-cell transfer (ACT) therapies, CAR-T therapy distinctly identifies tumour antigens through T-cells expressing specific chimeric antigen receptors (CARs) on their membrane, independent of the recognition pathways of major histocompatibility complexes (MHCs). The CAR architecture comprises three key components: an extracellular targeting domain for antigen recognition typically housing a single chain variable fragment (scFv) , and intracellular signal transduction domains encompassing TCR signal transduction molecules (usually CD3ζ) and co-stimulatory domains, these domains are connected with transmembrane domains consisting of small amino acid segments anchoring the CAR to cell membranes [2,3].

Currently, immune cells undergo gene modification through CAR transduction, including T cells (CAR-T), macrophages (CAR-M) and NK cells (CAR-NK) [4]. Among these immune cell types, the remarkable advancements of CAR-T cells have been proved by the clinical achievements, not only in hematological malignancies but also in demonstrating therapeutic potential against solid tumours.CAR-T cell therapy is becoming more and more clinically supported as a therapeutic option with promising futures. However, limitations persist in CAR-T cell therapy application, characterized by constrained treatment scope, challenges in ensuring sustained therapeutic effects, and clinical complications that cannot be overlooked [1].

Despite the promising advancements in CAR-T cell research, their clinical application remains constrained by various factors, hindering their evolution into a fully mature standard therapy. Regarding efficacy, addressing antigen loss, a longstanding challenge in targeted therapies remains imperative.The accomplishments of this line of inquiry center on identifying novel targets that are accessible and refining existing targets in order to better tailor CAR-T cell designs for the future. Furthermore, the performance of this novel therapy using engineered T-cells in solid tumour

treatment remains suboptimal, primarily attributed to unsuccessful efforts in identifying suitable antigen targets and inadequate tumour infiltration [4]. From a safety standpoint, mitigating targeted/off-target toxicity in clinical practice is essential, necessitating standardized clinical response strategies and treatment protocols to manage the heightened risk of complications. These intricate problems underscore the pressing need for further investigation and advancement of CAR-T cell therapy in order to resolve associated complications and improve its therapeutic effectiveness. Therefore, optimizing the efficacy and safety in clinical treatment of this kind of therapy has become a promising research direction to further enhance clinical application [1].

2. The Development and Typical Design of CAR-T

There have been five generations of CAR-T cell research to date, although other experts disagree, arguing that there have only been four generations. However, their definitions of CAR-T design for the first three generations are the same [1,3]. Firstly, a novel type of T cells was created by combining with just a single chain variable fragment (scFv) and CD3ζ. Despite their ability to activate T cells in vitro, these cells exhibited limited efficacy co-stimulatory signals absence, resulting as reduced T cell activity and persistence. As a result, their clinical applicability was constrained, rendering the first-generation CAR-T cell design a significant but unsuccessful endeavor. Subsequent advancements in CAR-T cell development have focused on enhancing and incorporating intracellular domains to bolster the functionality of these innovative immune cells. The incorporation of co-stimulatory domains such as 4-1BB or CD-28 into the intracellular signal transduction domain heralded the emergence of the second-generation CAR-T cells. These cells showed encouraging clinical results and made a substantial contribution to the advancement of this therapy. Third-generation CAR-T cells further evolved by integrating multiple structural domains to enhance anti-tumour activity and circumvent tumour escape mechanisms. The design purpose of fourth-generation CAR-T cells was to modify and enhance T cell function.This involves incorporating cytokine expression domains with the intracellular signal domain to facilitate cytokine secretion, thereby bolstering immune responses and enhancing therapeutic efficacy. Such advancements underscore ongoing efforts to refine CAR-T cell therapy, focusing on optimizing T cell functionality to combat cancer more effectively [3]. At present, some studies modify other components of CAR, such as transmembrane domains or targeting domains, so there are also views that this belongs to the new generation, i.e. the fifth-generation CAR-T cells. The researchers have successfully created new structures using nanobodies or DARPins to replace scFv as extracellular targeting domains [1,2].

3. CAR-T Cells in Cancer Therapy

Since the inception of the first CAR-T cells experiment, a plethora of this new therapy has garnered acceptance and approval from the FDA for various cancer types, including diverse hematological tumours and multiple myeloma. In clinical practice, the production of T cells with CAR expression commences with collection and purification of the T cells obtained from donors (allogeneic) or patients (autologous). Subsequently, self-inactivating lentiviral vectors or other biomedical methods such as γ-retroviruses, transposons, or CRISPR/Cas9 are employed to transfer engineered CAR genes into activated T cells. Presently, the substrates utilized for CAR transduction are not confined to specific subsets of T cells, CD8 and CD4 as well as other T cells are widely used by researchers in the design. And the optimization of various T cell products represents a burgeoning research focus. There are also exploration aims to refine and enhance the efficacy of this developing new therapy by leveraging diverse T cell populations and optimizing their characteristics [5].

Tisagenlecleucel (tisa-cel) stands as the first pioneering CAR-T cell therapy sanctioned for refractory/relapsing B cell acute lymphoblastic leukemia (ALL). As the first CAR-T drug to be launched, it was approved by the FDA in 2017. In the global Phase II ELIANA trial reported in August 2017, pediatric and young patients showed an overall rejection rate of 81%, with 59% of

respondents showing no recurrence. In the 3-year follow-up, the overall survival was 63%, and the 3-year relapse-free survival rate was approximately 50%, demonstrating the effectiveness and long-term safety of the treatment method [6].

Similarly, Idecabatagene vitamin (ide-cel), employed for managing refractory or relapsing multiple myeloma, approved by FDA in March 2021. Noteworthy outcomes from the preceding KarMMA trial showcased an 8.8 months median progression-free survival among 128 patients, coupled with a commendable complete response rate of 33%. In the subsequent follow-up conducted in February 2022, the complete response rate was 82.5%, illuminating the broadened horizons and promising application potential of this therapeutic intervention [5].

4. Current limitations of CAR-T

4.1. Antigen Loss in Treatment

Antigen loss denotes the complete absence or cessation of expression of the targeted antigen on tumour cells due to selective pressure exerted by the immune system or targeted therapies like CAR-T cells. The mechanisms underlying antigen loss are multifaceted, encompassing gene mutations, epigenetic modifications, and immune evasion mechanisms, all of which impact the selection of tumour cells. These mechanisms contribute to the evasion of immune surveillance and pose challenges for effective targeted therapies [1]. Antigen loss is believed to be associated with treatment failure and relapse, it affects long-term response rates by impairing the sustained efficacy of CART cells, leading to survival rates much lower than expected. In different types of cancer, the rate of antigen loss is various. Compared with other haematological malignancies, acute lymphoblastic leukaemia (ALL) has a higher probability of antigen loss and a higher recurrence rate after CART cell therapy, reaching 10% -57% [7].

Presently, an emerging strategy involves refining CAR-T cell design to target multiple antigens with reduced susceptibility to antigen loss, a goal achieved through thorough exploration of tumour heterogeneity. Although it demands plenty of time and finance to thoroughly identify and validate each targets antigen needed to create dual-targeted CAR-T cells, the targeting of redundant proteins holds certain promise in bolstering the stability of this target therapy and mitigating the impact of single mutations on treatment efficacy. Additionally, researchers are actively seeking more optimal target proteins that exhibit broader expression across tumour cells and are less influenced by tumour heterogeneity. For example, preclinical trialsin the treatment of B-cell ALL with bispecific CAR-T cells that target both CD22 and CD19 used have yielded promising results [8].

4.2. The Influence of Immunosuppressive TME

The production of cytokines such as IL-10 and IL-4 during the course of cancer drives tumour-associated macrophages (TAMs) to transition into the M2 phenotype. Moreover, the immunosuppressive tumour microenvironment (TME) induced by cytokines is attractive to mesenchymal derived suppressor cells (MDSCs) and regulatory T cells (T-reg cells). T cell cytotoxic function against tumour cells is decreased in this environment, while tumour cell motility and proliferation are enhanced [9]. Treg cells present in the TME exert suppressive effects on cytotoxic T lymphocyte antigen-4 (CTLA4) through the release of IL-2 and other signaling molecules, result in the inhibition of antigen-presenting cells (APCs), further diminishing T-cell activation and cytotoxic T-cell function. Moreover, MDSCs impede CAR-T cell functionality within the TME by targeting effector T cells. It is demonstrated that CAR-T cells exhibit more pronounced anti-cancer effects in patients with lower MDSC levels [10]. Tumour cells, especially those in solid tumours, produce lactate through anaerobic respiration. The accumulation of lactic acid in TME inhibits T cell signaling, T-reg cell expansion, and M2 macrophage differentiation, complicating the immune response within the tumour [11]. The modification of TME has been a hot research direction of cancer treatment research filed in recent years. Many strategies have been proposed to address immunosuppressive TME in CAR-T design, such as CAR-T cells modification in order to express

immune stimulatory molecules and regulate the expression of cytokines in TME, result as increasing immune stimulatory cytokine expression, or reducing immune suppressive cell activity. In efforts to enhance the efficacy of the CAR-T related immune therapies. scientists have tried to manipulate the genetic makeup of the T cells for CAR transduction to enhance their responsiveness to immunosuppressive cytokines like TGF-β, thus bestowing them with resilience against these inhibitory signals [9]. Furthermore, in the field of CAR-T cell design, it has been shown that the production of some bacterial-derived proteins can induce the activation of native immune cells. One example of such a protein is multipotent proinflammatory neutrophil activator protein (NAP), which is generated from Helicobacter pylori. This activation increases the treatment's effectiveness and strengthens the immune system's defense against tumour. Furthermore, cancer-associated fibroblasts (CAFs) assume a critical role in shaping the extracellular matrix (ECM) and contributing to its formation within the tumour microenvironment (TME). As such, CAFs targeting CAR-T cells are engineered to possess the capability of inhibiting extracellular matrix (ECM) development in the TME. Through the use of a focused strategy, they are able to overcome obstacles that physically impede the movement of CAR-T cells, allowing them to infiltrate and function within tumor tissues [11].

Indeed, an approach involves integrating other treatment modalities with the application of CAR-T cells, leveraging synergistic effects to enhance therapeutic outcomes. These combined strategies encompass a variety of approaches, including immune checkpoint inhibitors, co-stimulatory molecules, small molecule targeted drugs and other clinical treatments. However, the design and validation cycle of combination therapy is long, and the increase in treatment content also increases the complexity of treatment plans and the appearance of unknown toxicity. The combination of CAR-T cell treatment with checkpoint blockade (CPB) has been proven to increase the functional duration of CAR-T cells. In particular, inhibiting PD-L1 expression on MDSCs has significantly enhanced the therapeutic results of these engineered T cells in experimental settings. Moreover, the use of MDSC-depleting antibiotics can further augment this effect [12].

4.3. CAR-T Cell Traffic and Drug Delivery in Tumours

CART cell therapy has broad application prospects in the treatment of multiple kinds of tumours represented by haematological tumours, but its therapeutic effect on solid tumours is still restricted by the ability of immune cell delivery. The TME in solid tumours hinders CAR-T cells from locating tumour cell locations and infiltrating into the tumour interior from the bloodstream with the function of high concentrations of immunosuppressive cytokines and chemokines [4]. Solid tumours contain a dense mesh and pore structure formed by a large amount of elastin and collagen fibers, which, together with the tumour vascular system, physically limit the migration of immune cells and hinder T cells from entering the interior of the tumour. Tumour endothelial cells (EC) in the inner wall of blood vessels can also specifically inhibit effector T cells to achieve immune suppression [13].

For improving the traffic in TME of CAR-T cells, regulating chemokines and their receptors is a research direction that has already achieved preliminary achievements. Overexpression of chemokines associated with immunosuppressive cells is an important feature of chemokine dysregulation in solid tumours. Chemokines can attract immune cells expressing corresponding receptors. For example, in non-small cell lung cancer, chemokine CCL2 recruits M2 macrophages by binding to its receptors, and in Hodgkin's lymphoma, CCL17 and CCL22 attract T helper-2 cells expressing CCR4 and regulatory T cells while excluding CD8 T cells lacking CCR4 expression [14].Therefore, the strategy that is currently in use is genetically altering CAR-T cells to create chemokine receptors expression on T cell membrane, thereby amplifying their migration to the tumour sites. The presence of these chemokine receptors facilitates the recruitment of those modified T cells to tumours, consequently heightening their anti-tumour effectiveness. For instance, incorporating CCR4 expression into CAR-T cells directed against CD30 has demonstrated augmented CAR-T cell migration and enhanced tumour suppression capabilities [1].

Certainly, a variety of delivery methods and tactics are necessary for practical application of CAR-T cell therapy across varied cancer types. Local administration of these immune cells entails direct injection of T cells into the tumour or the cerebrospinal fluid, fostering increased T cell activity and bolstered anti-tumour effectiveness, especially notable in patients with glioblastoma. Research on local delivery methods for T cells is ongoing, aiming to optimize this approach for various cancer types [1]. To obtain persistent anti-tumor effects, CAR-T cell platforms with sustained release represent another advance. Examples include hyaluronic acid hydrogel and nickel titanium alloy solid biological scaffold, which serve as carriers for CAR-T cells, facilitating their gradual release into the tumour microenvironment. By maintaining CAR-T cells within the site of the tumor for a longer amount of time, these sustained-release devices hope to increase the anti-tumor activity over time. These developments in delivery techniques might potentially increase the efficacy of CAR-T cell therapy and broaden its range of cancer kinds of use [15].

5. Clinical Disorder

5.1. Toxicity and Syndrome

Indeed, toxicity in normal human cells have been reported in clinical treatment of CAR-T cells like many cancer treatment modalities. Cytokine release syndrome (CRS) stands out as the predominant adverse reaction linked to CAR-T cell therapy. Cytokines, such as interleukin-6 (IL-6), interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α), among others, cause a systemic inflammatory response that gives rise to CRS [1]. The majority of CAR-T cells target non-tumor-specific antigens, which can be harmful to normal cells (both on- and off-target toxicity). The targeting effect of designed T cells on somatic cells can cause inflammation and even severe tissue damage. The release of liquid contents from dead target cells containing cytokines during the lysis of tumour cells, along with the cytokines secreted by CAR-T cells after activation, jointly increases cytokines concentration in the internal environment, including multiple contents such as TNF- α, IL-6 and IL-10. Those cytokines will induce the production of CRS and cause an acute immune response [10]. During CAR-T cell therapy, immune effector cell-associated neurotoxicity syndrome (ICANS) is an additional serious side effect. Neurological symptoms and diseases of the central nervous system (CNS), such as aphasia, seizures, local dysfunction, and other neurological deficiencies, are its defining characteristics. That can be a result from the abnormal dissemination of the cytokines and T cells into the CNS. The consensus criteria established by American Society for Transplantation and Cellular Therapy (ASTCT) categorized ICANS and CRS into four grades based on the severity of symptoms and their impact on patient health. CRS grade>2 was reported in 10-30% patients with CAR-T treatment, presenting with fever accompanied by hypotension (≥ 38.0℃), requiring nasal intubation or other oxygen supplementation to alleviate hypoxia symptoms, and may require vasopressor drugs (≥3 grade). ICANS grade>2 was reported in 12-30% patients, manifested as aphasia (≥2 grade), epileptic seizures (≥3 grade), and even loss of consciousness and inability to wake up (4 grade) [16]. The researchers found that anti-CD19 CAR-Ts can transport across the blood-brain barrier (BBB), then they finally proved the existence of CAR-T cells in patient cerebrospinal fluid. Cerebrospinal fluid cytokines and T lymphocytes are thought to have a role in the development of ICANS, however the precise processes are yet unknown [17].

For isolated CRS, anti-IL-6 receptor tocilizumab is currently the preferred drug in clinical treatment, for IL-6 has the highest concentration in CRS models. In cases when tocilizumab fails to manage the CRS, corticosteroids such as IV dexamethasone provide an alternate strategy. If tocilizumab and corticosteroids both have a limited impact, IL-1 receptor antagonist anakinra and IL-6 antagonist siltuximab will be taken into consideration. However, the isolated CRS is rare in clinical, ICANS is tightly associated with CRS in clinical. The treatment focus of ICANS is on the treatment of seizures, such as corticosteroid treatment. Anakinra and siltuximab are considered possible treatment options, but the current evidence is still insufficient [16]. Neutralizing granulocyte-macrophage colony-stimulating factor (GM-CSF) with lenzilumab is prospect for managing adverse

reactions. There are studies demonstrated that the neutralization of GM-CSF with lenzilumab can mitigate neuroinflammation and CRS, particularly in acute lymphoblastic leukemia (ALL) patients.

5.2. CAR-T Cell Therapy Clinical Complications

In the CAR-T cell clinical application, there are numerous short-term or medium to long-term complications, including infections, tumour lysis syndrome (TLS), sepsis, and B cell regeneration disorders. After the CAR-T treatment, the fungal (<5%), viral (5-10%), bacterial (20%) infections are common in prognosis of patients. Most of these infections manifest as lower respiratory tract infections and occur within the first 10 days. TLS is a metabolic emergency caused by the dissolution of multiple tumour cells, leading to the entry of cellular contents into the systemic circulation. It manifests as hypocalcemia, hyperphosphatemia, hyperkalemia, and can further lead to acute renal failure, epilepsy, and even sudden death. Anti-CD19 CAR-T cells target the CD19 antigen, a kind of protein expressed in B cells, so patients receiving medium to long-term treatment may experience B cell regeneration disorders or hypogammaglobulinemia (25%). When it comes to other CAR-T cells targeting proteins expressing in B cell, this complication also appears [16].

For complications, intravenous injection of immunoglobulin or subcutaneous injection of immunoglobulin is the standard method for treating hypogammaglobulinemia. At present, the treatment strategy for B cell regeneration disorders is still mainly natural recovery, waiting for the number of B cells to recover and stopping immunoglobulin administration. In clinical trials, the dosage of corticosteroids used, and antibiotics applied during immune reconstitution is currently the focus of clinical research on reducing complications after CAR-T treatment and improving overall survival rate [16].

6. Conclusion

In conclusion, CAR-T cell therapy offers a targeted immune therapy with better efficacy and easier control of adverse reactions, symbolize a paradigm shift in cancer treatment. However, challenges persist particularly in addressing antigen loss and overcoming the immunosuppressive TME prevalent in solid tumours. Promising strategies, including dual-targeted CAR-T cells and TME modulation, offer avenues for enhancing therapeutic outcomes. Moreover, optimizing CAR-T cell trafficking especially in solid tumours remains a pressing concern, necessitating continued research into chemokine-based strategies. Combination therapy may be a clinical strategy to address some issues, but its complexity and potential risk factors need to be considered before designing a combination therapy plan.

Furthermore, mitigating toxicity and managing complications, such as CRS and infections, are critical for ensuring the efficacy and safety of CAR-T therapy. Current approaches, such as tocilizumab for CRS and immunoglobulin therapy for hypogammaglobulinemia, provide valuable insights but require further refinement. Additionally, long-term monitoring and management of medium or long-term complications, including tumour lysis syndrome and B cell regeneration disorders, are essential for improving patient outcomes.

Looking ahead, the development of CAR-T cell treatment will continue, yet also demands rigorous exploration and innovation. Anticipated advancements include the future generation CAR designs, the enhance of delivery strategies and the standardization of supportive cares.

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