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Analysis of the principles and development history of CAR T cell immunotherapy

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Abstract. With the increasing causes of cancer and the improvement of cancer diagnosis rates, the global burden of cancer is becoming increasingly severe. But at the same time, researchers are also gaining a better understanding of cancer and accumulating more and more experience in fighting cancer, which has improved the survival rate of cancer. The treatment method that modifies T cells with CAR genes to have anti-cancer effects has been named CAR T cell immunotherapy by researchers. This immunotherapy significant achievements have been made in the field of hematology treatment. tumors, and the related drug Kyrmiah was approved for market in 2017. This article briefly explains the basic principles of CAR T cell immunotherapy by integrating textual materials, and reviews and summarizes the development history and main achievements of this immunotherapy. By summarizing and organizing, exploratory learners quickly and directly understand the basic information of CAR T cell immunotherapy. Hope to help capable scholars accumulate relevant knowledge, discover and solve new problems discovered in the practical application of this therapy in the future, expand its application scope, reduce treatment costs, and enable more cancer patients in need of this technology to receive assistance.

Keywords: Hematologic malignancies, CAR T cells, immunotherapy, anticancer treatment.

1. Introduction

With the rapid development of the times and technology, people's living environments are becoming increasingly complex, lifestyles are becoming more diverse, and cancer is being exposed more and more frequently. In the history of human struggle against cancer, more and more new technologies have been developed. Chimeric antigen receptor (CAR) T-cell immunotherapy is a individual tumor treatment technique that has made breakthrough progress in recent years. This technology is mainly achieved through the synthesis of chimeric antigen receptors. The researchers first extracted T cells from the patient's body, genetically modified them, and ultimately made them express anti-cancer genes, becoming chimeric antigen receptors. Researchers need to expand and activate CAR T cells to help patients' T cells have the ability to specifically recognize specific antigens on the surface of cancer cells. Then, the patient's somatic cells have the ability to target and kill cancer cells. T-cell immunotherapy is currently widely used to combat hematological malignancies such as lymphoma and acute lymphoblastic leukemia [1].

The development of CAR T-cells can be traced back to the 1980s.During this period, scientists discovered that T cells in the immune system have special functions. They can recognize and attack

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cancer cells. This discovery has laid a very important foundation for subsequent scholars to study immunotherapy [2]. In 2011, American cancer researchers showed the significant effect of CAR T-cell therapy in the treatment of ALL in their study, which became a milestone in the development of this immunotherapy, and this therapy appeared in the vision of more cancer researchers. Since then, major pharmaceutical companies have invested in supporting the research and development of such therapies. In 2017, the US Food and Drug Administration (FDA) approved the market of Kyrmiah, the world's first CAR-T product for the treatment of relapsed or refractory B-cell precursor ALL, which ushered the tumor therapy into a new therapeutic stage of modifying immune cells with cells [2]. With the development of the times and technology, more and more advanced science and technology are being used by researchers to conduct in-depth research on CAR T immunotherapy. In order to compensate for the shortcomings of this immunotherapy, CAR T cells have undergone iterative upgrades from the first to the fifth, improving the efficiency of these immune cells and achieving better therapeutic effects.[3]

Researchers have conducted in-depth research step by step, witnessing and promoting the entire process of CAR T-cell immunotherapy from basic scientific discovery to clinical trials and FAD approval. Researchers have always attached great importance to the clinical performance of this immunotherapy, hoping to improve the efficiency of CAR T cell preparation and better control its side effects through research. Although drugs have been approved, the frequent occurrence of cytokine immune syndrome (CRS) and the side effects of neurotoxins associated with treatment still accompany this immunotherapy. The practical problems of high treatment costs and insufficient durability of treatment are also waiting to be solved.

Researchers hope to continuously improve CAR T-cell immunotherapy through research, enhance its efficacy, reduce its side effects, expand its application scope, and prolong its duration of action. In addition, researchers are constantly striving to enrich the individualized approaches of CAR T cell immunotherapy, hoping to provide more efficient and accurate treatment for each patient with different tumor characteristics and immune system status.

This article will briefly introduce the basic principles of this immunotherapy and review its development history. Formatting the title, authors and affiliations

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2. The underlying logic of CAR-T cell immunotherapy

CAR is a genetically modified and synthesized modular antigen receptor with antibody characteristics and efficient TCR activation signal [4]. It possesses an antigen-binding domain and a signal transduction domain. When people want to initiate CAR T immunotherapy, they first need to collect T cells from blood samples of healthy individuals or patients. These T cells will be designed and assembled into CAR-T cells by introducing CAR genes. Then these CAR gene modified T cells can bind to and activate specific tumor surface antigens when they find them. One advantage of this type of T cell is that its mutual recognition with tumor cells is not limited by the typing of human leukocyte antigen (HLA) [4]. The assembled CAR-T cells will be expanded in vitro and finally infused back into the patient to affect the tumor microenvironment by releasing proinflammatory cytokines such as interferon-γ (IFN-γ) and interleukin-2(IL-2), and recruit endogenous immune cells to kill the tumor. Use its acquired specific anti-tumor ability to help patients fight tumors [4]. The transmembrane domain is not a very well characterized region compared to other domains. Most transmembrane domains are derived from native proteins, including CD3ζ, CD4, CD8α or CD28 [5]. The primary function of this region is to anchor the CAR to the T-cell membrane. In addition, some studies have found that signal transduction or synapse formation are active and dimerize with endogenous signals, thereby affecting the expression level and stability of CAR [5].

The Signaling Domain is responsible for activating T cell function. It usually includes one or more signal transduction molecules, such as the CD3ζ chain used to transmit activation signals and other costimulatory molecules such as 4-1BB, CD28, etc., which enhance the activation and proliferation of T cells.

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3. The development history and major achievements of CAR T-cell immunotherapy

3.1. Clarify the CAR structure

AS a modular antigen receptor, CAR consists of four major components. In addition to the antigen-binding and signaling domains already mentioned above, CAR also has a linker region and a transmembrane domain.

The portion of the CAR that gives the target antigen specificity is called the antigen-binding domain. Monoclonal antibodies' varied heavy and light chains are the source of the antigen-binding domain, which are linked by flexible connectors to form a single-chain variable fragmant (scFv). Several properties of this scFv allow simple recognition and binding of target epitopes and influence CAR function. For example, the mode of interaction between variable heavy and light chains and the relative position of complementation determining regions can affect the affinity and specificity of a CAR for its target epitope [5]. This affinity fundamentally determines CAR function and is a particularly important antigen-binding domain parameterr. This value must be high enough to support CAR recognition of antigens on tumor cells, induction of CAR signaling, and activation of T cells. However, the value of affinity should not be so high that it will cause activation and induced death of CAR-expressing T cells and cause toxicity.

Extracellular structures that extend the binding unit from the transmembrane domain are referred to as hinge or spacer regions. The hinge's function is to lengthen the antigen-binding domain so that it can make contact with the target epitope and to give flexibility to get over steric hindrance [5]. Crucially, it seems that the hinge that is selected influences CAR function, since variations in the hinge region's length and makeup impact signaling, CAR production, flexibility, activation output intensity, and epitope recognition [5].

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3.2. The five generations development of CAR T experience

According to the different intracellular signal transduction structures, CAR-T cells have developed to the fifth generation. The first-generation cars, which were designed in the late 1990s, contained CD3ζ or FcRγ signaling domains [5]. The vast majority of these cars depend on activating CAR-T cells through immunoreceptor tyrosine activation motifs derived from CD3ζ. Nevertheless, signals from these motifs alone are incapable of generating effective T cell responses, as evidenced by the poor durability and persistence of the first-generation CAR in vitro [5]. Clinical studies have also confirmed these findings, but the results have shown limited or no efficacy [5].

In an early in vivo model of B-cell malignancies, the importance of co-punctibility for CD-19-targeted CAR T cells to eat well was demonstrated [5]. On the basis of an understanding of the importance of co-spictivity for durable CAR T-cell cell therapy, a second-generation CAR was designed with one additional intracellular signaling region than the first. The second-generation CAR includes a costimulatory domain, derived from CD28 or 4-1BB, located between the transmembrane and CD3 signaling regions [4]. At present, second-generation CAR is the most widely seen CAR T cell product that has been approved and marketed by the FDA. In clinical applications, second-generation CAR-T cells have demonstrated significant therapeutic effects in various hematological malignancies, including acute lymphoblastic leukemia (ALL), B-cell acute lymphoblastic leukemia, diffuse large B-cell lymphoma, and multiple myeloma. Currently, the efficacy of second-generation CAR-T cells in solid

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tumors is still under investigation, with related diseases including glioblastoma, advanced sarcoma, liver metastases, mesothelioma, and pancreatic cancer, the generation of CAR reflects two co-stimulatory regions in one structure compared with the second generation [5].

On the assumption that costimulation through only one domain would result in incomplete activation, a third-generation CAR that concatenated two costimulatory domains in a single construct, combining two costimulatory domains in tandem with CD3ζ38, was produced. However, the progress in preclinical studies of third-generation cars has not been as promising as expected. The progress is that compared with the second generation CAR39, CAR combining CD28 and 4-1BB signaling leads to stronger cytokine production in lymphoma, and lung metastases show improved antitumor responses in vivo [5]. Third-generation cars did not show in vivo therapeutic benefit in leukemia and pancreatic cancer models and failed to outperform second-generation cars in their respective models.

Vehicles that improve T-cell activity are sometimes referred to as "fourth-generation cars" by researchers, particularly if they contain more receptors or create more protein molecules like cytokines. These CARs are also known as T-cell pick-up for universal cytokine killing (TRUCK) CARs [4]. This generation of CAR-T is equipped with the ability to integrate other stimulatory domains on the basis of the second and third generation of CAR and co-express some key cytokines or costimulatory ligands, aiming to modify the tumor microenvironment, for example, the release of pro-inflammatory cytokines like CD40L, IL-18, or IL-12 to improve antigen cross-presentation and encourage epitope diffusion. [6].

Comparing these four generations of CAR T cells together, the reason why second-generation CAR is the most common and commonly used CAR T cell product on the market is that it has stronger anti-tumor ability than the first generation, but it does not have the more adverse reactions caused by the strong killing power of the third and fourth generations.

The fifth-generation CARs, which are in testing, contain an IL-2 receptor β fragment (IL-2Rβ). IL-2Rβ replaces OX-40/CD27 and can mediate tyrosine kinase production and signal transducer and activator of transcription 3/5 activation, but the effectiveness and safety of the fifth generation CARs are in the verification stage [7,8].

3.3. Breakthrough of CAR T in the treatment of hematological malignancies

ALL is a malignant clonal disease of lymphoid precursor cells with the highest incidence rate among children's tumors. With the treatment of CART immunotherapy, it reduces the recurrence problem of most adults due to chemotherapy resistance.At present, two commercial CAR-T cell products, tisa-cel and brex-cel, have been approved for children and young adults under 25 years old and adult B-ALL, respectively. The rate of minimal residual disease (MRD)-negative complete remission (CR) was 81% in the former group, and 71% in the latter group [1].

The first CAR-T cell product approved for lymphoma treatment is CD19 CAR-T cell axi-ce in ZUMA-1(NCT02348216), which is mainly used to treat patients with refractory relapsed diffuse large B-cell lymphoma (R/R LBCL). Analysis of long-term follow-up 5-year overall survival data, 42.6%, proved that this product has a relatively good therapeutic effect [1]. The phase III ZUMA-7(NCT03391466) trial compared the safety and efficacy of Yescarta with the current standard of care in second-line treatment of adults with R/R LBCL. The results showed that CAR-T cell therapy was superior to standard therapy, including autologous transplantation [1].

Multiple myeloma(MM) is a malignant disorder marked by abnormal proliferation of plasma cells accompanied by increased synthesis and secretion of monoclonal immunoglobulin or its polypeptide chain subunits. CAR-T cell therapy has achieved remarkable efficacy in MM, making it from a tumor with low treatment response rate to a disease with diverse treatment methods, treatable and controllable [1]. In the KarMMa(NCT03361748) trial, Ide-cel achieved an overall response rate (ORR) of 73% and a stringent CR(sCR) rate of 33%.

4. Conclusion

This article briefly introduces the basic principles of the work of T cells under the modification of CAR gene in patients, the development history and major achievements of CAR T cell immunotherapy. This

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kind of immunotherapy holds considerable promise in this era of increasing causes of cancer, increasing diagnoses, and substantial global cancer burden. It has taken nearly half a century for researchers to discover that T cells can recognize and attack cancer cells, to genetically modify T cells to show remarkable efficacy in the treatment of hematologic tumors, and to obtain FDA approval for this type of treatment. This article hopes to make a brief introduction to CAR T and outline the development of CAR T cell immunotherapy, so as to provide learners who have just learned about this immunotherapy with a direct and rapid preliminary understanding of this immunotherapy, and to facilitate interested readers to accumulate background knowledge when conducting in-depth research on CAR T immunotherapy.It is hoped that in the future, these exploratory researchers will continue to find and solve the problems of CAR-T cell immunotherapy in laboratory research and clinical application, and continue to expand the application scope of this immunotherapy, such as trying to improve its application in solid tumors. At the same time, it is hoped that researchers and pharmaceutical companies can make more efforts in cost control in the future, such as strengthening international cooperation and knowledge sharing to jointly address the issue of treatment costs, exploring new payment methods such as pay per efficacy, pay per disease stage, or pay per treatment effect, so that CAR T immunotherapy can be truly promoted to the public and used in those who have a real need for this excellent immunotherapy.

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