CAR-T: A Promising Cell Therapy for Cancer Treatment

The past decade has seen significant advancements in chimeric antigen receptor T (CAR-T) cell therapy. As evidenced by a growing number of successful clinical trials and FDA approval, CAR-T therapy has emerged to revolutionize cancer treatment.

Since 2017, the FDA has approved six CAR-T cell therapies used to treat blood cancers such as lymphomas, leukemia, and multiple myeloma. A 2022 report revealed approximately 2,754 active cell therapy agents in the global immunotherapy pipeline, a 36% increase from the number reported in 2021. CAR-T has the most significant number of pipelines and ongoing trials of all immunotherapies, with 1,432 pipelines and 857 trials worldwide, indicating that it remains the most active and promising immunotherapy (Fig.1) [1].

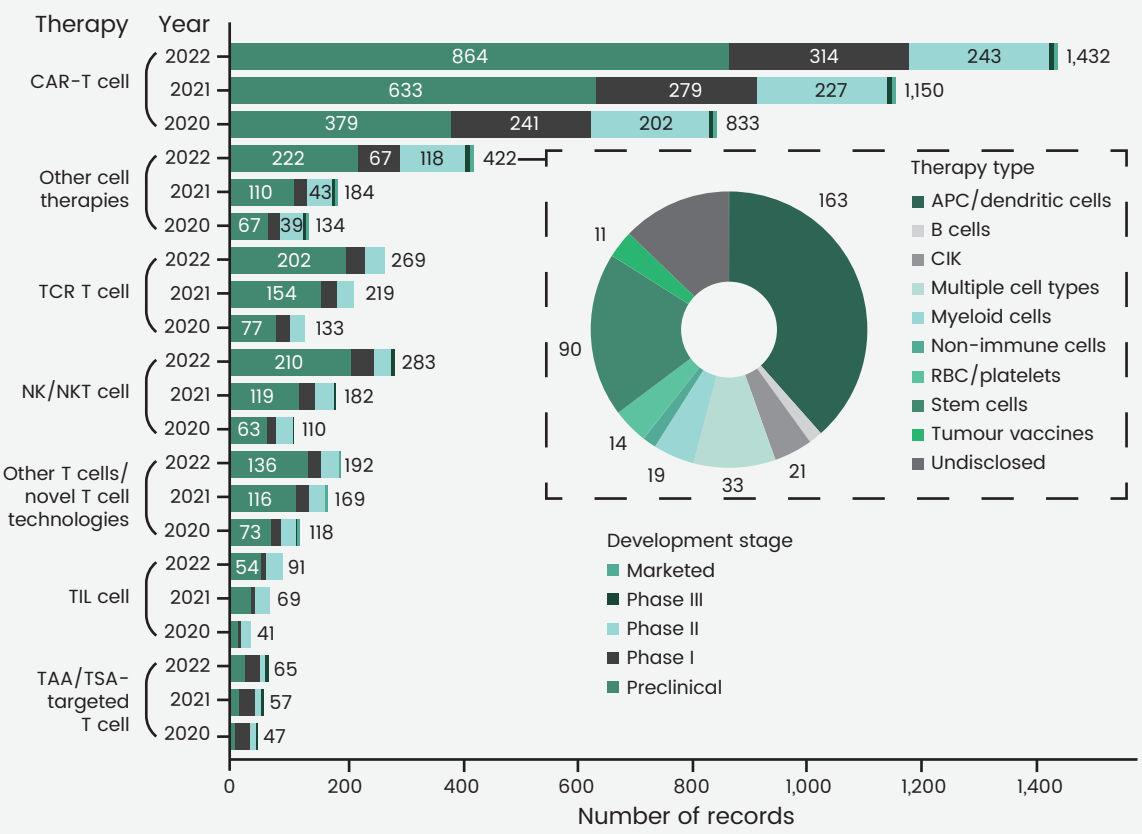

Fig. 1: Changes in the cancer cell therapy pipeline by therapy type and year. Comparison of cell therapy agent development pipeline across various therapy types from 2020 to 2022. APC, antigen-presenting cell; CIK, cytokine-induced killer; NK, natural killer; RBC, red blood cell; TAA, tumor-associated antigen; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; TSA, tumor-specific antigen. The pie chart shows the composition of the 'other cell therapies' category in 2022. *Source: https://doi.org/10.1038/d41573-022-00095-1*

The sequential production stages include the collection and expansion of patients' T cells; CAR molecule design, screening, and transfection; target selection; and quality control (Fig.2). Here, we explore the key steps involved in CAR-T production to provide insights into the challenges that may impede successful CAR-T therapy.

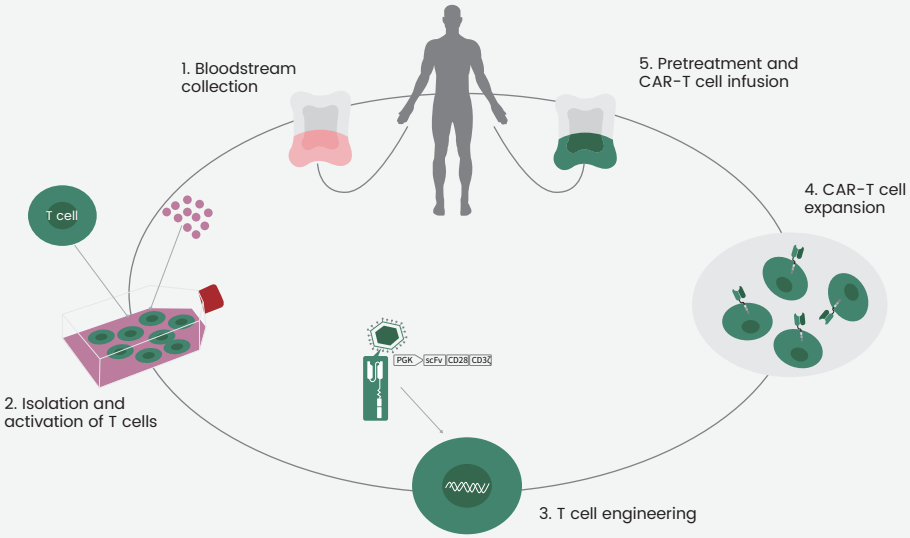

Fig.2. CAR T-cell Therapy Procedures

CAR-T Cell Activation and Expansion

CAR-T is a type of immunotherapy that uses a patient's own T cells to treat cancer by harnessing the power of the patient's immune system to target and kill cancer cells. The first step in CAR-T production is collecting T-cells from a patient. As the number of cells is limited, it requires expansion to a certain amount (billions to even tens of billions of CAR-T cells) before infusion back to the patient, making CAR-T cell expansion an essential step during development. Notably, cytokines can stimulate CAR-T cell activation and expansion. For example, IL-2, IL-4, IL-15, IL-7, and IL-21 are widely used in CAR-T cell preparation and culturing[2].

Because cytokines are used in CAR-T cell culturing, the purity or grade of cytokines and the level of remaining cytokines in the final product require strict monitoring and control to avoid any potential risks to the patient's health. Therefore, choosing high-quality cytokines and reliable cytokine detection methods, such as cytokine ELISA kits, is becoming increasingly important in CAR-T development.

CAR Molecule Design

The CAR molecule guides the CAR-T cells to the target on the cancer cell surface and activates them into cancer-killing agents. Therefore, the CAR molecule must recognize the cancer antigen specifically and precisely to target cancer cells while avoiding healthy cells effectively. In addition, to provide a better recognition ability for CAR-T cells, the CAR molecule should be effectively designed, as it affects the durability and persistence of the CAR-T cells in the body.

A potent CAR molecule should have several key features: high specificity for the target antigen, efficient activation of CAR-T cells, and reduced off-target effects. In recent years, extensive research efforts focused on optimizing the CAR molecule have enabled its transition from a first-generation to a fourth-generation design (Fig.3)[3].

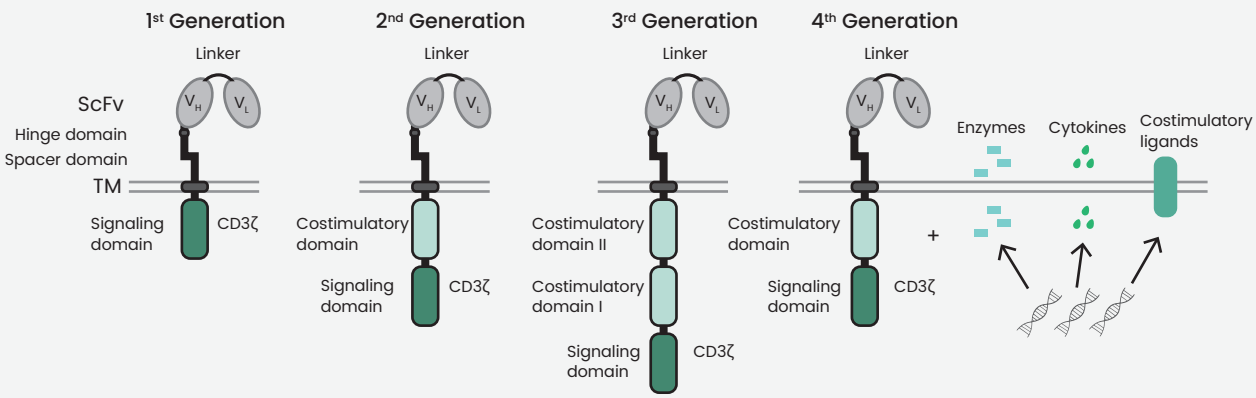

Figure 3. Chimeric antigen receptor generations. The 1st generation CART cells induced T cell activation only by the primary signal via the CD3ζ signaling domain. CART cells were further developed by integration of a costimulatory domain in 2nd generation CART cells. The 3rd generation CART cells consist of two costimulatory domains. The future 4th generation CART cells combine the vector with enzymes, cytokines, and costimulatory ligands.

Source: doi:10.3390/ijms20246223

The five typical components of the CAR model are explained below.

Antigen-binding domain: This domain controls recognition and binding to the target antigen on the surface of cancer cells. This is typically an antibody fragment such as a single-chain antibody fragment (scFv). Screening the scFv molecule efficiently and conducting reliable affinity tests are the most significant steps in CAR molecule development, which will affect CAR-T therapy efficacy and safety.

Costimulatory domains: These domains control CAR-T cell activation and expansion. They typically comprise signaling domains, such as CD28 or 4-1BB, that provide a secondary signal to the CAR-T cells to stimulate activation and expansion.

Linker region: This region connects the antigen-binding and costimulatory domains. It regulates signal transmission between the two domains and is critical for the proper functioning of the CAR molecule.

The transmembrane domain: This domain connects the extracellular and intracellular domains of the CAR-T cell, which anchors the CAR molecule to the CAR-T cell surface. This domain must be stable, as it maintains the correct orientation of the CAR molecule.

Cytokine domains: Cytokines can enhance CAR-T cell activation. For example, IL-2 can stimulate cell proliferation and maintain cell viability during expansion[4]. Therefore, some CAR molecules may also include cytokine domains that secrete cytokines, which stimulate the immune system and enhance CAR-T cell activation and expansion.

CAR-T Drug Target Selection and Validation

Because CAR-T presents a better capability to treat hematological malignancies than solid cancer, drug targets for hematological malignancies, such as CD19, BCMA, and CD22, remain the most-used target proteins, according to a 2022 report[1].The fact that four of the six FDA-approved CAR-T therapies use CD19 as a target and the other two use BCMA indicates that these targets are significant and feasible. Given the competition among these hot targets, other novel targets, such as GPRC5D, CLEC12A, CD7, and CD3, have emerged in recent years, all of which hold promise for uncovering the potential for novel CAR-T discoveries.

Unlike hematological malignancies, solid tumors have a more complicated microenvironment, which limits the ability of CAR-T cells to reach and penetrate tumors. Therefore, drug targets for solid tumors require careful selection and screening. The most widely used drug targets for solid cancer include PSCA, HER2, MSLN, EGFR, MUC1, and PSMA.

During CAR-T production, drug targets are used for scFv screening and discovery as well as for CAR-positive rate detection. In CAR-T therapeutic products, target cells (CAR-positive T cells) that can correctly express both CAR and T-cell surface markers should be selected for detecting the proportion of target cells. CAR-positive T cells are active components that play a role in tumor killing. Therefore, the positive rate of CAR transfection is a necessary test item for the product quality control of CAR-T cells. Notably, flow cytometry (FCM) remains a widely used technology in the development of CAR-T cell therapies, especially for identifying the phenotype and functionality of CAR-T cells. Therefore, the availability of high-quality reagents suitable for FCM, such as various fluorescence-labeled drug targets and corresponding antibodies, is a critical factor that could facilitate CAR-T development.

Challenges and Future of CAR-T Cell Therapy

Owing to several FDA approvals, CAR-T-cell therapy remains the most promising cell therapy for patients with hematological malignancies in comparison to other CAR-engineered cell therapies, such as CAR-NK and CAR-M. However, some challenges must be addressed to guarantee the real success of CAR-T therapy in cancer treatment. First, collecting T cells from patients can be difficult and time-consuming, especially for patients with cancer who have a compromised immune system and very few T cells. Second, the use of CAR-T to treat solid cancers is not as effective as for hematological cancer due to the complex and heterogeneous microenvironment of solid tumors, which prevents CAR-T cells from reaching and infiltrating tumors. Finally, the cost and availability of CAR-T therapy limit its utilization because CAR-T cells, which are created specifically from patients, are highly individualized, more expensive, and require a longer production period than other immunotherapy-based methods.

Despite these challenges, researchers are still actively exploring different strategies to make CAR-T therapy effective against solid tumors and more commercially viable.

As the world's leading supplier of bioreagents and CRO services for the biopharmaceutical field, Sino Biological provides comprehensive solutions for CAR-T cell therapy development. Our reagents and services serve clients through each stage from early target discovery to preclinical research and development.

Reference:
1. Landscape of cancer cell therapies: trends and real-world data. https://doi.org/10.1038/d41573-022-00095-1
2. Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. https://doi.org/10.1182/blood-2014-01-552174
3. The Application of CAR-T Cells in Haematological Malignancies. doi.org/10.1007/s00005-020-00599-x
4. The Role of Checkpoint Inhibitors and Cytokines in Adoptive Cell-Based Cancer Immunotherapy with Genetically Modified T Cells. doi: 10.1134/S0006297919070022

Sino Biological US Inc. (U.S.A.)
Tel: +1-215-583-7898
Email: order_us@sinobiologicalus.com

Sino Biological Europe GmbH (Europe)
Tel: +49(0)6196 9678656
Email: order_eu@sinobiologicaleu.com

Sino Biological, Inc. (Global)
Tel: +86-400-890-9989
Email: order@sinobiological.com

株式会社日本シノバイオロジカル (Japan)
電話番号：044-400-1330
ご注文：order@sinobiological.co.jp