TAMING THE T CELL

Joseph Martin and Tristan Free explore the latest developments in the T-cell-based therapy CAR-T and the challenges that need to be overcome for them to become common clinical practice.

As genetic engineering techniques have become more sophisticated over the last decade, our ability to manipulate cells for a specific purpose has advanced. Nowhere has this been more clearly demonstrated in the last few years than by the T cell.

The rise of the much-lauded chimeric antigen receptor (CAR) T-cell therapy has been ably assisted by gene-editing techniques such as CRISPR and zinc finger nucleases [1]. These techniques have allowed researchers to integrate ever more intricate structures and adjustments into T cells in order to fine tune the therapy.

Despite the encouraging rate of development in these therapies, many challenges remain in their commercialization and implementation while characterization remains an evolving area.

CAR-T CELLS: ADDRESSING THE CHALLENGES

CAR-T therapy relies on the incorporation of CARs into a patient's extracted T cells. The key components of these CAR molecules are the single-chain variable antibody domain (scFv), which can be designed to target specific tumor-associated antigens (TAAs), and the intracellular signaling domain, primarily consisting of the CD3 ζ chain of a T-cell receptor (TCR). On binding to the TAA, the CAR initiates proliferation of the CAR-T cells and the death of tumor cells through the release of cytokines, bypassing the need to bind to antigens presented by the tumor's multi-histocompatibility complex (MHC) [2].

SURVIVING IN HOSTILE TERRITORY

From the inception of CAR-T, the hostile tumor microenvironment (TME) has been established as a challenge to the therapy, reducing the survival and proliferation of CAR-T cells. Over the past few years, the basic formula of the therapy has been updated to include the current fourth generation of CARs. Each evolution has been designed to address barriers such as the TME and to increase the potency of T cells.

The initial evolution included the addition of a costimulatory domain to the CAR complex, such as CD28 or CD137 [3]. These second-generation CARs display an increase in T-cell proliferation, anti-apoptotic protein and cytokine release [4]. The third generation simply introduced a further costimulatory signal, while the fourth involves additional stimulation of transcription factors to induce the production and secretion of specific cytokines and interleukins in order to promote the potency of T-cell action [5]. The scope of these additional functional transgenic proteins in the fourth generation does not end with the simple amplification of T-cell potency. These proteins could be selected to provide cells with an on/off switch or a suicide gene to enable greater, more granular control of the therapy [1].

To further promote CAR-T cells as a viable therapeutic option capable of surviving in the TME, researchers have developed dual receptor CAR-T cells that contain the traditional TAA targeting CARs and an additional receptor for cytokine-mediated growth stimulation helping to offset the effects of the immune checkpoint signals in the TME [5].

The next generation of CAR-T cells is hypothesized to include the knockout of the T cell's human leukocyte antigen alongside TCR genes, allowing T cells taken from healthy donors to be used and avoiding their rejection by the cancer-patient host, therefore enabling their application in multiple patients [1].

TACKLING TOXICITY

A further challenge in CAR-T is dealing with the toxicity of the therapy. The typical toxicities observed with CAR-T are cytokine release syndrome (CRS), neurotoxicity and brain edema. As these cells target tumor surface proteins, unrestricted by the MHC and are engineered to be vehemently potent, the off-target effects of the treatment have the potential to be devastating. As the ligands found on the surface of tumors can often be found in healthy, ►

Figure 1. Demonstrating the different binding modalities of (A) classical CAR-T cells, (B) simultaneous and (C) sequential UIRs [5].

functional cells in different regions of the body, the selection of an appropriate target is vital. This is perhaps most painfully exemplified by the first in-human use of third-generation CARs that had been designed to target HER2 [6]. Minutes after the administration of 10^{11} HER2-targeted CAR cells, the patient experienced extreme pain and entered a comatose state from which they did not recover, dying 5 days later. It is likely that the natural presence of HER2 in pulmonary epithelial cells was a significant factor in the observed accumulation of CAR-T cells in the patient's lungs [5].

To address this pressing concern, researchers are designing universal immune receptors (UIRs) for use in CAR-T cells that can target multiple TAAs. Unlike standard CARs, these UIRs contain an additional extracellular adaptor domain that connects intracellular T-cell signaling domains to a soluble tumor antigen targeting ligand (TL) (Figure 1). In this instance, T-cell activation is induced by cell binding to the 'tag' or binding domain on the TAA-bound TL. This enables post-translational alteration of the T cell's target specificity by alteration of the soluble TL [7].

This development means that if the T cells begin to elicit a cytotoxic response in an undesired region of the body, they can be retargeted by changing the TL. Additionally, if the tumor evades initial administration due to tumor heterogeneity or subsequent

evolution, the engineered T cells can be retargeted to TAAs on the newly evolved tumor cells [7].

CAR-T CHARACTERIZATION

Due to the complexity of live products, variability in starting material and difficult characterization, CAR-T cell manufacturing is considered to be much more challenging than that of traditional biologics. CAR-T cell therapy manufacturing entails several carefully performed steps from lymphocyte collection to genetic modification with the CAR construct. These complex steps, alongside the requirement of reagents and source materials with differing complexity, are all key elements that must be completely understood to ensure that the product has the correct identity, potency and purity. Therefore, product characterization is key for all successful biological drug development.

Breaking down the manufacturing framework, it is important to identify chemical, biological and physical attributes, known as critical quality attributes, that can be measured continually.

Ramello *et al.* recently addressed the uncertainty involved in CAR-T cells' mechanism of action, specifically how CARs interact with endogenous T-cell molecules. This characterization is essential to provide tools to develop novel receptors with more tightly controlled functions [8].

CAR-T COMMERCIALIZATION

The early stage of this field, when combined with the complex manufacturing that gene-edited cell therapies require, means that their price is particularly expensive. Two recently approved CAR-T therapies – Yescarta and Kimriah – are currently priced at ~US\$373,000 and ~US\$475,000, respectively [10].

The manufacturing process is obviously a key driver behind the price tag. However, this is not so straightforward to fix. First, the manufacturing process must result in a safe and clinically effective cell product for the patient. Second, the process must be robustly reproducible, which is a necessity to validate it and to ensure quality. So, if the logistics are improved, yield increased and supply chain streamlined, then surely that would help reduce the cost of these therapies?

In order to increase the robustness and scalability of the bioprocesses to fit the therapy's needs, companies such as Oxgene are accelerating the discovery and production of cell and gene therapies by addressing key industry bottlenecks and facilitating new therapeutic approaches [11].

An automation equipment company, ThermoGenesis, is also attempting to solve the abovementioned problems through the development of CAR-TXpress™, a platform designed to automate many of the manual steps involved in cell processing. Platforms such as CAR-TXpress are helping strive towards more readily available CAR-T cell therapies by reducing processing time and increasing cell recovery, in order to significantly reduce the overall manufacturing time and cost, and increase the yield and efficiency of high-value samples [12].

Many people believe there are still some issues surrounding the production of cell therapies, but it is clear that the situation is improving. Non-gene-edited allogeneic CAR-T therapy or 'off-the-shelf' technology could make CAR-T therapy significantly cheaper by removing the need for individualized manufacturing, thus removing a big burden from healthcare systems. Do people need to buy into the concept of an autologous therapy?

The alloSHRINK Phase I trial, Celyad's trial of CYAD-101 combined with chemotherapy for the treatment of metastatic colorectal cancer, has thus far yielded promising results, with no severe toxicity reported [13]. As promising as this technology is, doubts still surround whether allogeneic CAR-T can really be an off-the-shelf therapy and more work is still required to overcome challenges with regards to immunology as well as manufacturing.

For the time being, schemes have been set up to ensure that patients can obtain these notoriously expensive treatments. The Advanced Therapy Treatment Centre (ATTC) project aims to develop robust systems for the routine delivery of cell and gene therapies to patients around the UK in the fastest and most cost-effective way possible [14]. For example, a number of payment schemes are being considered, including one that involves spreading the payment out over time to better share the risk of the therapies failing.

FUTURE PERSPECTIVE

CAR-T therapy will continue to develop technically in a step-wise, almost evolutionary fashion. As issues such as toxicity,

off-target effects and T-cell survival arise and are countered with ever more sophisticated solutions, new unforeseen challenges are sure to happen. However, with each evolution these challenges should grow less dramatic and inhibitory to the application of CAR-T cells. The current exciting rate of development bodes well for the theoretical application of these cells in a wide variety of settings.

Challenges for the development of a more controlled and cost-effective manufacturing process have however yet to be overcome. Despite there only being a few therapies approved and on the market, there is a growing number of institutes engaging in CAR-T research and manufacturing. With the increased interest and cell therapy still largely being in the research and development phase, standardization and characterization will become critical. If the manufacturing problems can be solved and the production process streamlined, consequently, the cost of these therapies should be reduced.

However, this is much easier said than done. It will take the cooperation between pharma, biotech academic institutes and official bodies (FDA, EMA, and so on) to ensure that the path towards safe and affordable therapies is accelerated.

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