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The future of transposon-based CAR-T cell production in immunotherapy

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Abstract. Chimeric Antigen Receptor (CAR) T-cell therapy, being one of the most critical treatment regimens for certain types of lymphomas, leukemias, and multiple myeloma, is experiencing ever-increasing demand. Its high expense and low availability have inflicted many issues among patients in need of this treatment. This paper aims to address these socioeconomic problems by concentrating on the future and current development of transposon-based CAR-T cell production in immunotherapy. Switching viral vectors to transposon-based vectors will greatly decrease the cost and manufacturing time of this personalized product. Transposon vectors also have advantages over viral vectors in sense of DNA carrying capacity and safety matters. Research organizations such as the CARAMBA Project are undertaking new transposon technology in the hope to increase the accessibility of CAR-T cell immunotherapies. Transposon-based CAR-T cell production may pave way for therapeutically and financially satisfying immunotherapies.

Keywords: CAR-T therapy, transposon-based vectors, viral vectors, immunotherapy.

1. Introduction

CAR-T therapy is essential in the treatment progress of relapsed leukemia patients. Currently, the CAR-T cell manufacturing process proceeds as follows: Firstly, a patient's white blood cells are extracted and collected via leukapheresis. Secondly, suitable T-cells are identified from the white blood cells and extracted to undergo CAR-T adaptation. Thirdly, to produce CAR-T cells, CAR gene sequences are introduced into the DNA of T cells [1]. With the use of engineering, CAR-T cells can recognize and target the SLAMF7 protein in myeloma cells. Lastly, mass personalized and affordable manufacturing of CAR-T cells occurs in labs. After an appropriate amount is produced, the T-cells are injected back into the patient, where they can proliferate and remove targeted cancerous cells. However, available and accessible clinical use of viral vector-mediated CAR-T therapy has been constrained by expensive costs and laborious production procedures [2]. Other drawbacks include immunogenicity, cytotoxicity, and increased risk of tumor development resulting from insertional mutagenesis [3]. On the contrary, transposon-based vectors are cost-effective, safe, and efficient. The high stability and transfer success rate of CAR genes are another benefit. Additionally, transposons feature an unlimited DNA-carrying capacity that exceeds that of viral vectors. With such potential, transposon-based CAR-T cell production has captured the spotlight of many projects aiming to widen the accessibility and lower the cost of CAR-T therapy. These projects and research pave the way for

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therapeutically effective, commercially appealing, and socioeconomically preferable CAR-T treatments [4].

2. Mechaniasm

2.1. Role of vectors in CAR-T cell therapy

In a nutshell, vectors are vehicles that help insert desired genetic material into the host cell. There are two main types of vectors, viral vectors, and non-viral vectors. In this paper, non-viral vectors will be discussed. In standard CAR-T cell immunotherapy, vectors are used to replace, change, or add a normal copy of a gene to nonfunctional or malfunctioning versions of the gene.

One of the essential features that make a vector useful and efficient is its genetic material-carrying capacity. While viral vectors fail to accommodate large amounts of therapeutic genetic material, transposon-mediated vectors have no limit in carrying capacity [5]. In other words, non-viral vectors are able to transfer and insert the same number of genes using less time and work than viral vectors. Transposon-based vectors deliver genes through a cut-and-paste mechanism in which a transposase enzyme integrates transposon DNA containing the desired transgenes directly into chromosomal DNA. Thus, transposon-based vectors greatly enhance the transposition success rate compared to the indirect transfer of naked nucleic acids (DNA and mRNA which cannot pass through cell membranes) through viral infections.

2.2. Introduction to the mechanisms of transposons

Transposons, also known as transposable elements or "jumping genes", are DNA sequences that are able to insert or excise themselves at DNA sequence sites. Transposable elements are categorized into two major classes according to their transposition mechanisms. Class I transposable elements, known as retrotransposons, require reverse transcription to transcript RNA back into DNA in order to transpose [6]. Class II transposable elements, known as DNA transposons, are able to transpose directly without transcription [6].

The "cut and paste" mechanism of DNA transposons can thus be manipulated to integrate specifically selected DNA sequences into the chromosomes of vertebrate animals in an attempt to alter current genes or introduce new traits [7]. Two of the main transposons discussed in this paper will be the Sleeping Beauty transposon and the piggyBac transposon; they are both DNA transposons. The Sleeping Beauty (SB) transposon system is a synthetic DNA transposon that can be used to insert distinct DNA sequences into a target area through transposition [8]. The piggyBac (PB) DNA transposon is characterized by a short-inverted repeat and, like the SB system, is able to transpose itself at target sites.

2.3. How transposon-based vectors function and help the production of CAR-T cells

As mentioned previously, transposon-based vectors have an unlimited genetic material carrying capacity compared to the limited amount viral vectors are able to carry. This would greatly reduce the time and cost of CAR-T cell production if transduction failure and frequency both decrease [9]. Transposons may replace viruses in playing the role of vectors in CAR-T cell production. Instead of the typical procedure of integrating desired DNA through manipulated viral infections, transposons are able to directly integrate the genetic material into the target cell's chromosomal DNA.

2.4. Mechanism of the CAR-T drug

The main objective of CAR-T therapy is to "train" the patient's malfunctioning T cells to reprogram them into cancer/tumor cell killers. This immunotherapy allows T cells to bind to target cell surface antigens via a single-chain variable fragment recognition domain. These modified T cells are then injected back into the patient to fight cancerous cells. The goal of non-viral vectors is to enhance the efficiency of the "training" of T cells and lower the cost for patients who require these newly trained cancer cell fighters [10].

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3. Application

3.1. Current CAR-T drugs

Three CD19 CAR-T cell products: Kymriah, with a lentiviral-based vector, Yescarta, with a retroviral-based vector, and Tecartus, also with a retroviral-based vector, are currently in usage and under further development. Non-viral transcription factors, however, are required for these drugs to become financially sustainable and easily accessed by patients in need. Details on these drugs will be presented below, and summarized in Table 1.

3.2. Kymriah

Kymriah harnesses the power of a patient's own T cells by modifying them with a CAR. It has a 4-1BB costimulatory domain which is in charge of promoting Kymriah's growth and persistence [11]. Kymriah binds to the protein CD19 which alerts the immune system to target cancerous cells. It is usually used in adult B-cell non-Hodgkin lymphoma or specific types of B-cell acute lymphoblastic leukemia. Side effects may include cytokine release syndrome, neurological toxicities, and serious allergic reactions. Kymriah costs approximately $475,000 per treatment course [12].

3.3. Yescarta

The implementation of Yescarta is similar to that of Kymriah, though it is usually used as a treatment for follicular lymphoma or for relapsed large B-cell lymphoma patients [11]. Side effects include hives, cytokine release syndrome, and other nausea or weakness. This form of CAR-T therapy aims to help the immune system defeat cancer cells. Yescarta costs approximately $373,000 per treatment course [13].

3.4. Tecartus

Tecartus is another type of CAR-T cell treatment that utilizes the patient's own T-cells to engineer a lymphoma cell-targeting entity. It is a potential treatment for adults with mantle cell lymphoma or acute lymphoblastic leukemia as a supplementary therapy whilst another is employed [14]. Common side effects include fevers above 100.4°F/38°C, low blood pressure, and dizziness. Tecartus costs approximately $373,000 per treatment course [15].

Table 1. Information of Kymriah, Yescarta and Tecartus.

Drug name	Application	Toxicity/Side effect	Cost per treatment course
Kymriah	Relapsed or non-respondent diffuse large B-cell lymphoma (DLBCL), relapsed or non-respondent follicular lymphoma (FL), and B-cell acute lymphoblastic leukemia (ALL)	Cytokine release syndrome, neurological toxicities, and serious allergic reactions	$475,000
Yescarta	Adult follicular lymphoma and relapsed large B-cell lymphoma	Cytokine release syndrome, nausea/weakness, and hives (allergies)	$373,000
Tecartus	Mantle cell lymphoma and acute lymphoblastic leukemia as supplementary treatments	Low blood pressure, fever higher than 100.4°F/38°C, and dizziness/fatigue	$373,000

3.5. The CARAMBA Project

The CARAMBA Project is one of the many research programs advancing in the direction of non-viral gene transfer. In fact, the CARAMBA Project conducted the first clinical trials prepared by non-viral

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Sleeping Beauty (SB) transposon gene transfer technology. Phase I and II clinical trials were conducted using patient-deprived T-cells and engineered to distinctly express an artificial CAR for myeloid antigen SLAMF7, which is identically expressed on all multiple myeloma cells [16]. That said, with the addition of virus-free SB CAR gene transfer from DNA minicircles to its cutting-edge technology, the CARAMBA project will pave the way for a therapeutically effective, commercially appealing, and socioeconomically preferable myeloma treatment. This will make SLAMF7 CAR-T cells an economically feasible and sustainable pharmacological product [16].

The innovative SLAMF7 CAR design enables CAR-T cell selection, detection, expansion, and deletion. To avoid immunogenicity and early rejection of CAR-T cells, the specific SLAMF7-targeting domain is humanized. The fact that SLAMF7 CARs have an EGFRt safety switch that may be activated by anti-EGFR antibodies to decrease CAR-T cells in the event of toxicity is a significant advantage and breakthrough in the field [16]. The CARAMBA project presents a high-level, virus-free CAR gene transfer into secure genomic loci and constitutes an important advance toward the creation of myeloma CAR therapy.

According to the CARAMBA Project's official website, their clinical trials proceed through the following steps. Firstly, a patient's white blood cells are extracted and collected via leukapheresis. Secondly, suitable T-cells are identified from the white blood cells and extracted to undergo CAR-T adaptation. Thirdly, to produce CAR-T cells, CAR gene sequences are introduced into the DNA of T cells. With the use of engineering, CAR-T cells can recognize and target the SLAMF7 protein in myeloma cells. Lastly, mass personalized and affordable manufacturing of CAR-T cells occurs in labs. After an appropriate amount is produced, the T-cells are injected back into the patient, where they can proliferate and remove targeted cancerous cells.

4. Future perspectives

4.1. Future advancements

Transposon-mediated CAR-T therapy is still under development and modifications, therefore there are countless areas where this technology can improve on. The application range of this drug is still limited to certain blood cancers, however, if the identification of target antigens is able to expand from the currently available CD19 antigen then the drug may be trialed in solid tumor contexts [2]. Another challenge of applying CAR-T to solid tumors is its difficulty in infiltrating physical barriers present in tumors. One possible solution is to engineer CAR-T cells to express certain enzymes that degrade tumor extracellular matrixes [17]. Even if the CAR-T product make it through the first obstacle, it would have to sustain functionality under hostile tumor immunosuppressive microenvironments. Utilizing combination therapy (combining check-point blockade (CPB) agents with CAR-T) or engineering CAR-T cells to emit stimulatory cytokines that promote antitumor activity and maintain function could be potential solutions to issues with functionality [17].

It is also necessary to determine whether transposon-based CAR-T cells will be as efficient and safe as viral-based ones in terms of insertional mutagenesis and transposon remobilization in order for future clinical trials to proceed [2]. Possibility of creating "off-the-shelf" CAR-T products in contrast to current personalized drugs is also an objective that could be considered or even reached.

4.2. Future perspectives

Transposon-based non-viral CAR-T cell production is a field with extensive potential. Not only does transposon-mediated CAR-T cell production lower manufacturing costs, but it also has a larger genetic material-carrying capacity. Current published resources point towards the utilization of the Sleeping Beauty system in future non-viral CAR-T cell production. With high hopes of producing inexpensive, safe, and efficient CAR-T treatments transposon-based CAR-T cells may be the next groundbreaking invention in the field of hematology.

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5. Conclusion

Transposon-mediated CAR-T therapy has the potential of decreasing the high costs of CAR-T products while enhancing manufacturing efficiency. Advancement of this technology will widen the availability of CAR-T therapy at an affordable price, contributing to healthcare and medical development.

Transposon-based CAR-T cell production is still in the stages of early clinical development. Nevertheless, the benefits of transposon vectors are already acknowledged and researched. From safe integration to limitless DNA-carrying capacity, non-viral transposon-based CAR-T production will push the limits of CAR-T treatment and serve as a novel weapon against cancer for patients in need. Transposon-based CAR-T cell production will pave way for a clinically successful, commercially appealing, and socioeconomically advantageous product.

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