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CAR T-Cell Research: Current Clinical Challenges and Outlook

Chimeric Antigen Receptor (CAR) T-cell therapy has revolutionized the field of oncology, producing exceptionally effective and durable clinical responses for some cancer types.

CARs are recombinant receptors engineered to redirect the specificity and function of immune cells. In CAR T-cell therapy, T-cells are genetically modified to express a CAR using genetic engineering tools such as viral transduction and CRISPR-Cas9. These modified T-cells target and bind to extracellular surface cancer antigens resulting in major T-cell activation and in turn, elimination of malignant cells through processes such as induced apoptosis and cytokine release[1].

The buzz surrounding CAR T-cell therapy has translated to a steep rise in the number of associated research papers, clinical trials, and approved drugs over the past decade. Nevertheless, major limitations to CAR T therapies prevent their widespread use in oncology treatment.

Here we review the main challenges, outline the research efforts underway to overcome them, and discuss the future direction of CAR T therapies.

Challenges associated with CAR T therapy starting materials

All current approved CAR T-cell therapies and the majority of published clinical trials have used autologous materials, which means the cells are derived from the patient for whom the therapy is being developed. However, therapies based on autologous T-cells are associated with several limitations. One major challenge, for example, is a dependence on the functional fitness of patient T-cells. And this is often diminished by the disease or prior therapies such as lymphodepleting chemotherapy and radiotherapy, which can make it difficult to obtain enough patient-derived, viable T-cells for development.

As an alternative, allogenic starting materials obtained from healthy donors yield high amounts of fully functional cells that can be used to generate multiple CAR T-cell products. While facilitating an 'off-the-shelf' therapy option, allogenic materials come with their own unique set of challenges.

The main limitation of allogenic CAR T-cell products is graft-versus-host disease (GvHD). This leads to host allorejection, which ultimately hinders anti-tumor activity and can potentially result in fatalities. GvHD arises when there is a mismatch between the donor and recipient human leukocyte antigens (HLA)[2]. In an attempt to overcome this issue, studies have worked on eliminating expression of T-cell receptors (TCR) in CAR T-cells using a range of gene editing tools; including Zinc Finger Nucleases (ZFN)[3–5], Transcription Activator-Like Effector Nucleases (TALEN)[6,7], and CRISPR-Cas9[8–10]. Although high gene-editing efficiencies have been achieved using these techniques, some TCR-bearing T-cells may persist that still have the potential to cause GvHD[11]. Alternative approaches to reduce the risk of GvHD and allorejection include the elimination of HLA molecules[12], and tumor site-specific activation strategies such as hypoxia-activated CAR T-cells[13].

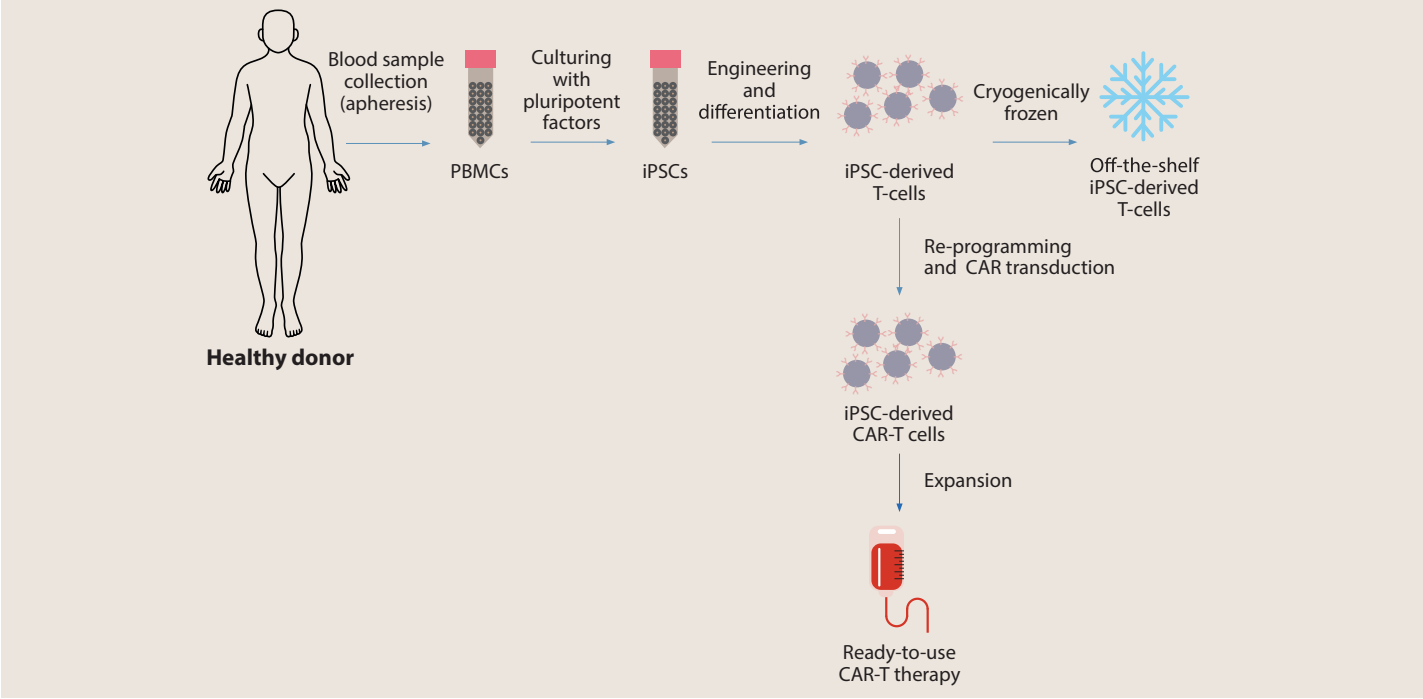

Fig. 1: Simplified workflow for the production of iPSC-derived T-lymphocytes. Briefly, peripheral blood mononuclear cells (PBMCs) are recovered from a healthy donor by apheresis. Culturing PBMCs with pluripotent factors yields iPSCs which can subsequently be engineered and differentiated to yield iPSC-derived T lymphocytes.

A general limitation to both autologous and allogenic CAR T therapies is the exhaustion of T-cell ability during their amplification – which is an essential step of CAR T therapy development[14]. Additionally, T-cells are notoriously difficult to edit, limiting their use with engineered antigen receptors[14]. T-cells differentiated from either hematopoietic stem and progenitor cells (HSPCs) and induced pluripotent stem cells (iPSCs) offer an opportunity to overcome these limitations (Fig. 1). Additionally, both HSPCs and iPSCs provide rapid access to unlimited, phenotypically defined, expandable and functional T-cells. This possibility is generating increasing interest in the use of HSPCs/iPSCs for CAR T therapies[15]. Engineered iPSC-derived T-cells expressing CARs have been generated, demonstrated therapeutic potential in model organisms[16], and recently, moved into Phase 1 clinical trials[17].

Lack of Efficacy for Treating Solid Tumors

The inability of CAR T-cells to traffic to disease sites, overcome physical barriers (e.g. tumor stroma) to infiltrate solid tumors, and maintain function in a hostile tumor microenvironment has so far limited their therapeutic potential to blood disorders. Indeed, all the current approved CAR T therapies are used to treat hematological malignancies.

One strategy to widen the scope of CAR T therapies is through engineering the expression of various proteins on CAR T-cells. For instance, CAR T-cells engineered to express heparanase, an enzyme that degrades the primary component of tumor stroma (heparin sulfate proteoglycan – HSPG), have been investigated as a method to improve tumor penetration[18].

Certain chemokines that are known to correlate with disease burden and prognosis in multiple solid tumors have recently been exploited to improve CAR T-cell trafficking. By engineering CAR T-cells to express chemokine receptors that recognize and respond to tumor-specific chemokines, various studies have shown enhanced trafficking and significantly improved antitumor efficacy both *in silico* and *in vitro*[19–22]. Whether these results translate *in vivo* is yet to be explored.

Even if CAR T-cells successfully traffic and infiltrate the tumor, the hostile and immunosuppressive environment of tumors can prevent T-cells carrying out their effector function. Weak or non-existent responses, poor T-cell expansion, and short-term T-cell persistence are all regularly observed when treating solid tumors with CAR T-cell therapy[22]. Again, genetically engineered CAR T-cells are at the center of research efforts. For example, so-called "armored CARs" secrete stimulatory cytokines that support the survival, proliferation, and antitumor activity of CAR T-cells[23].

Alternative methods to manage the tumor microenvironment include combining CAR T-cell therapy with checkpoint blockade (CPB) agents. Through this combination therapy, CAR T-cells are able to infiltrate tumors while CPB agents reverse CAR T-cell inhibition to aid sustained T-cell persistence and function[24]. Furthermore, the exploration of alternative antigen recognition domains to mitigate T-cell exhaustion is underway. Single-chain variable fragments (scFvs) are typically used to mediate antigen recognition but are linked to T-cell exhaustion. Engineered binding scaffolds, natural ligands, or receptors offer promising alternatives[25].

CHALLENGES	SOLUTIONS
Trafficking/migration Complex human physiology make it difficult for CAR T-cells to home in and migrate onto malignant sites	Engineering CAR T-cells to express proteins that aid migration, such as adhesion molecules and/or chemokine receptors that match and respond to tumor-derived chemokines
Infiltration Physical barriers can prevent or limit CAR T-cell infiltration of a solid tumor	Engineering CAR T-cells that express enzymes capable of degrading the tumor extracellular matrix
Maintenance of function Hostile and immunosuppressive environment of tumors can hinder T-cells carrying out their effector function	Engineering of CAR T-cells to secrete stimulatory cytokines that support the survival, proliferation, and antitumor activity as well as rebalance the tumor microenvironment. Combination therapy: combining CAR T-cell therapy with checkpoint blockade (CPB) agents.

Antigen Escape

Antigen escape is used to describe the partial or complete loss of target antigen expression on malignant T-cells in patients treated with CAR T therapy. It is a common mechanism of resistance that allows malignant cells to evade CAR T-cells. This phenomenon is responsible for the high rate of post-therapy relapse in patients treated with CAR-based therapies and is therefore an important focus for current research.

Engineering CAR T-cells that can recognize multiple targets is being explored as a solution to overcome antigen escape. These methods either use dual CAR constructs, tandem CARs, coadministration, or cotransduction (Fig. 2). Both dual CAR constructs and tandem CARs have shown promising efficacy in clinical trials, decreased antigen escape, and in some cases have even shown favorable safety profiles[22,26].

Most CAR T-cells for blood disorders are engineered to recognize CD19, an antigen expressed by malignant cells in leukemia patients[27]. However, the ability to recognize multiple targets hinges on the identification of novel antigens that are suitable targets for CAR T therapies. Moreover, suitable targets for solid tumors are scarce since antigen expression is highly heterogenous and many tumor antigens are also expressed on healthy cells, risking off target effects[28]. There are many potential novel targets under investigation including some which offer opportunities to develop new therapeutic options. For instance, targeting antigens on cancer stem cells that drive tumor growth offers a promising anti-tumor therapy[29].

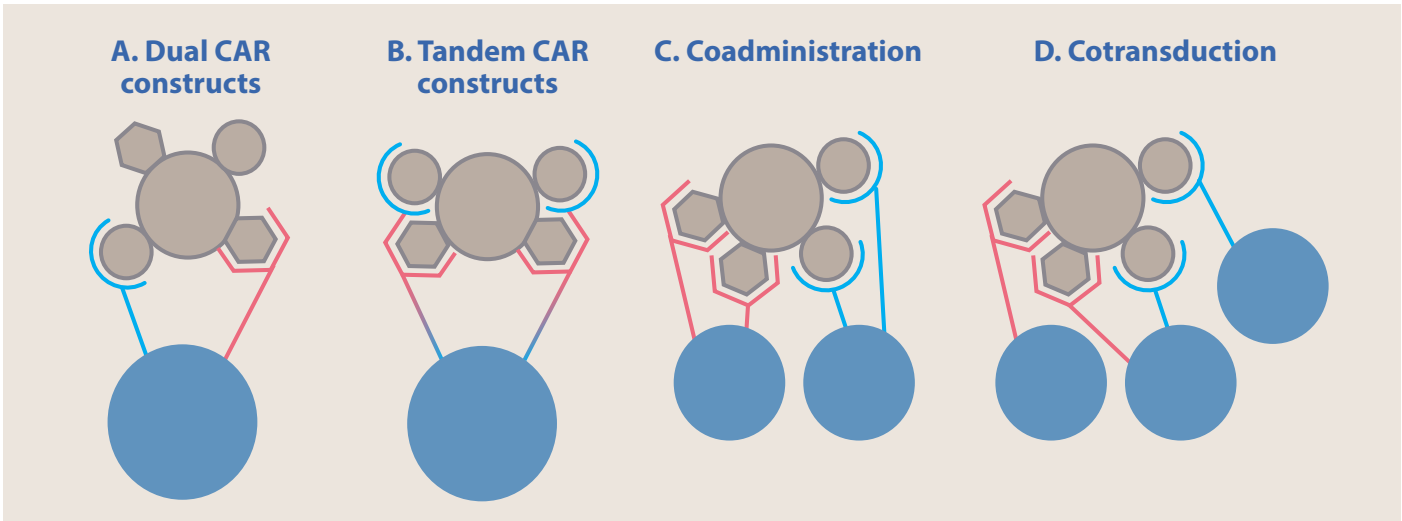

Fig. 3: . Strategies for engineering CAR T-cells to recognize multiple targets.

Summary

CAR T therapies are predominantly used after first-line treatment has failed. For instance, in the UK, CAR T therapies are vailable to children and young adults with B-cell acute lymphoblastic leukemia (ALL), whose first treatment has not worked[30]. However, there is considerable interest in utilizing CAR T-cell therapy earlier in the course of treatment.

While CAR T therapies have already revolutionized the treatment of certain hematological malignancies, there are still limitations to their implementation and therapeutic effect. Currently, approved therapies rely on autologous material that is often insufficient for CAR T-cell manufacture due to the health status of the patient. Even with successful CAR T-cell delivery, malignant T-cells can downregulate antigens due to selective pressures.

In solid tumors, getting CAR T-cells to traffic to and infiltrate solid tumors, penetrate physical tumor barriers and survive in the immunosuppressive tumor microenvironment is a significant challenge. While these challenges exist, CAR T-cell research is increasingly delivering novel strategies and potential solutions that drive forward more effective and safer therapies, as well as expanding the types of treatable malignancies.

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