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Are We There Yet? How CAR T-Cells Came to Be and Where They'll Take Us

Kate Fitzsimmons[1]*

[1]Simon Fraser University, *Department of Biological Sciences*

Abstract

Chimeric Antigen Receptor (CAR) T-cells have shown immense promise for the treatment of blood cancers. Fundamentally, CAR T-cell therapy involves the redirection of the immune system's natural response against pathogens towards the body's own cancer cells. The structure of the CAR allows the circumvention of the major histocompatibility complex, thereby allowing CAR T-cells to exhibit toxicity toward a chosen antigen. Advancements in CAR structure have improved CAR T-cell expansion and potency, also giving rise to a subset of engineered T-cells that can deposit cytokines into solid tumours. However, at this time the overall scope of CAR T-cells as a therapy is limited. Solid tumours are difficult to treat with CAR T-cells due in part to lack of appropriate target antigens, physical barriers to their efficacy, and a hostile tumour microenvironment. Toxicity is also an impediment to their clinical application. In this review, the molecular and physiological basis of CAR T-cells is outlined and areas for future research are briefly explored.

Keywords — Cancer, Chimeric Antigen Receptor T-Cells, Immunotherapy, Tumor Microenvironment, Toxicity

1. Introduction

CAR T-cells are genetically engineered T-cells designed to attack the cancerous cells of the patient from whom they are derived. To manufacture them, the patient's T-cells are modified outside the body to contain an antibody-derived receptor, such that the cytotoxicity of the T-cell can be directed towards any tissue that expresses the corresponding antigen. As of 2018, only two CAR T-cell therapies are available on the market. Both are intended to treat relapsed or refractory cancers - those that have either returned after previous improvement or have not responded to conventional treatment. Tisagenlecleucel (Kymriah), which is specific for the antigen CD19, is used in the treatment of B-cell precursor acute lymphoblastic leukemia (ALL) in children and young adults [1]. Axicabtagene ciloleucel (Yescarta), also specific for CD19, has been shown to be effective in the treatment of large B cell lymphoma in adults [2]. Both have shown promising short-term results: in an international Phase 2 study of tisagenlecleucel, 83% of patients experienced overall remission within 3 months after a one-time infusion, with overall remission being defined as complete remission with or without complete hematologic recovery that lasted for at least 28 days and was confirmed by laboratory tests [1]. A Phase 2 trial of axicabtagene ciloleucel had similarly notable results. After at least 6 months, 54% of patients had experienced complete response, and another 28% had experienced partial response [3].

*Corresponding Author: kfitzsim@sfu.ca

2. The Immune System

When a foreign body is recognized by the immune system, the innate immune response is activated. The term "foreign body" generally refers to external pathogens that an individual may encounter but, in the case of cancer, the desired target of the immune response may be the body's own cancerous cells. The innate immune response is so called because it consists of a set of genetically programmed responses towards such foreign particles [4]. This reliance on genetics imposes an important limitation: due to the finite number of genes that can be coded for in the human germline, the specificity of the innate immune response is lacking [5]. If the innate immune system is overwhelmed, the adaptive immune response is activated [4].

There are several important varieties of cells that constitute the adaptive immune system. The effector cells of the adaptive immune response are known as lymphocytes [4]. Sub-lineages of lymphocytes include B-cells and T-cells [4]. When they encounter a foreign body, B-cells secrete soluble immunoglobulins known as antibodies [4]. Conversely, the receptors of T-cells are restricted to the cell surface and are known, simply, as T-cell receptors [4]. The ligands of antibodies and T-cell receptors are known as antigens [4].

One essential distinction between the innate and adaptive immune responses is how foreign bodies are recognized. Antibodies and T-cell receptors are not derived directly from the genome, but rather from genes that undergo recombination and modification during lymphocyte development [4]. These receptors are therefore not bound by the genetic constraints that the receptors of the innate immune response face and can be made in billions of versions [6]. Each of these unique receptors can bind just one ligand, therefore allowing lymphocytes to recognize foreign bodies with a high degree of specificity [4].

However, T-cells, the vehicle for CARs, face their own limitations. A properly functioning immune system can distinguish "self" cells from "non-self" cells, a principle in part carried out by major histocompatibility complex (MHC) molecules. MHC molecules display fragments of proteins from a cell, either endogenous or pathogen-derived, on the cell's surface in a peptide-MHC molecule (pMHC) complex [7]. Developing T-cells first encounter MHC molecules in the thymus, where they present the T-cells with a variety of self-derived peptides [8]. If the T-cells bind to the self pMHC complexes with high specificity, they are instructed to perish, as they pose the rise of coordinating an attack against the body's own tissues [8]. Conversely, T-cells that bind to the self pMHC complexes with low to medium affinity will survive [8]. This selection allows vast variation within the body's T-cell population while minimizing the risk of autoreactivity [8]. While this distinction between self and non-self is vital for healthy immune function, it prevents standard cytotoxic T-cells from destroying the body's own cancer cells. However, CAR T-cells can harness the cytotoxicity of T-cells for this very purpose by bypassing the MHC. Rather than recognizing pMHC complexes using a T-cell receptor, the addition of a CAR allows direct recognition of antigens due to the structure of the receptor. CARs have four main parts: an extracellular, antibody derived domain used for antigen recognition; a transmembrane domain; a spacer or hinge domain; and an intracellular domain that plays a role in T-cell activation [9]. By using an extracellular domain of antibody origin, the CAR can recognize any cell-surface

antigen for which there is a corresponding antibody, including but not limited to those associated with MHC molecules. Furthermore, these antigens that CARs recognize can comprise many different structures, from peptides to inorganic compounds to carbohydrates, as opposed to T-cell receptors, which recognize exclusively peptides [10].

3. CAR T-Cell Structures

The two CAR T-cell therapies currently on the market, tisagenlecleucel and axicabtagene ciloleucel, are second-generation CARs of a four-generation series. T-cells with chimeric receptors that allowed antibody-type specificity in recognition of antigens were developed by Kuwana et al. in 1987, and in 1993 Eshhar et al. directed these principles towards cancer cells, calling the modified T-cells "T-bodies" [11, 12]. T-bodies, now known as first-generation CARs, utilize a CD3ζ T-cell activation domain [12]. The cytotoxic effect of these CAR T-cells is lacking due to their inability to produce sufficient levels of interleukin-2 (IL-2) [13]. As such, to achieve therapeutic results, exogenous cytokines are administered concurrently [14]. Cytokines, such as IL-2, are a general class of small, soluble molecules that influence how cells interact with each other [15]. IL-2 specifically plays a role in T-cell expansion, a process in which T-cells proliferate and differentiate from naïve T-cells into effector T-cells capable of cytotoxicity [16]. Clinical data suggests that the efficacy of CAR T-cells is influenced by the infused cells' ability to expand in vivo [17]. The magnitude of the T-cells' expansion is also related to the number of T-cells that will persist after the pathogen is eradicated, known as memory T-cells [18]. Therefore, an added benefit of treating cancer using T-cells is that any memory T-cells generated also have the potential to provide long-term tumour immunosurveillance against the cancer [19]. Due to the vital role that IL-2 plays in the expansion of CAR T-cells, the need to supply exogenous cytokines is unfavourable if a different CAR design could eliminate this requirement. Krause et al. accomplished this in 1998 by modifying T-cells to express a CD28-like receptor as a costimulatory domain that recognized a ganglioside found on the surface of many tumours [20]. These modified T-cells, now known as second generation CARs, were able to secrete their own IL-2 [20]. For modern second-generation CARs, CD28 or 4-1BB are often the costimulatory domains of choice [21]. This costimulation confers stronger persistence and therefore more potency in general [22]. Research performed by Savoldo et al. in which first- and second-generation CAR T-cells with the same antigen recognition domain were administered to the same patient showed that second-generation CARs demonstrate improved expansion and persistence relative to first-generation CARs [23]. Third-generation CARs, following the logic that one costimulatory domain notably improves the performance of the engineered T-cells, contain two costimulatory domains [24]. More research is warranted on third-generation CARs. So far, results in mouse models have been promising [25]. However, early human trials have shown lackluster results [26].

4. CAR T-Cells and Solid Tumours

The fourth-generation of CARs are specifically aimed towards solid tumours [27]. The need for a specific subset of CARs able to treat solid tumours is due to the many barriers that T-cells face when it comes to treating cancers outside the realm of hematologic malignancies. A primary challenge is identifying tumour-associated antigens (TAAs) suitable for attack by CAR T-cells. Such TAAs must fulfill two crucial criteria: their expression must be consistent on the surface of tumour cells, but low or absent on normal tissues [28]. Part of the success of CAR T-cells in treating B-cell malignancies such as those targeted by tisagenlecleucel and axicabtagene ciloleucel can be attributed to the TAA that is targeted: CD19. CD19 is expressed on both cancerous B-cells and non-cancerous B-cells, meaning it violates the latter of the two criteria for TAAs outlined above [28]. Attack of non-cancerous cells can lead to B-cell aplasia, the depletion of B-cells in the patient's blood [29]. Although the expression of CD19 on non-cancerous cells is not optimal in terms of its quality as a TAA, the corresponding aplasia can be counteracted via intravenous administration of a mixture of antibodies, known as intravenous immunoglobulin (IVIG) [29]. While the mechanism by which IVIG supports the immune system is not well understood, it involves processes such as interaction with the cytokine network, activation of B- and T-cells, and provision of otherwise depleted antibodies [30]. Overall, the administration of immunoglobulin likely acts to recapitulate the role of antibodies in the homeostasis of a functioning immune system [30].

The search for solid tumour TAAs has been intensive and remains ongoing. TAAs that have undergone exploration in clinical trials include Human Epidermal Growth Factor Receptor 2 (HER2), carcinoembryonic antigen, the diganglioside GD2, and interleukin 13 receptor α [28]. In addition, a recently published Phase I trial in which epidermal growth factor receptor (EGFR)-specific CARs were used was notable as far as CAR T-cell therapy for solid tumours goes, with ten out of seventeen participants achieving stable disease following infusion [31]. Although some success has been seen in trials focused on the aforementioned TAAs, the broad applicability of CAR T-cells for solid tumours is still limited in part by the identification of appropriate CAR targets.

The use of CAR T-cells for the treatment of solid tumours also presents a more literal barrier to their efficacy. As opposed to the case of hematologic malignancies where the bloodstream contains an admixture of both cancerous cells and T-cells, in the case of solid tumours CAR T-cells must navigate from the blood to the tumour site in a process known as trafficking. Chemokines, which are cytokines that induce chemotaxis, play a role in recruiting lymphocytes to the tumour [32]. For instance, chemokines such as exodus-2 and macrophage-derived chemokine are found to be expressed at higher levels in ovarian tumours containing T-cells than in those without [32]. Ideally, chemokines expressed by the solid tumour recruit lymphocytes with the corresponding chemokine receptor, however in some cases the chemokines produced by the tumour do not correspond to receptors on the T-cell [33]. In addition, the expression of chemokines involved in migration of effector cells varies among tumours, and in some cases the amount of ligand produced is not enough to promote efficient trafficking of effector cells [34]. To some extent, this heterogeneity in the chemokines produced as well as their variable amounts can help account for the inefficient trafficking of leukocytes to

the tumour site [35]. Other structural aspects of solid tumours also prevent lymphocyte trafficking. In normal tissue, pericytes support the endothelial tissue of capillaries and venules, however in tumours they are often loosely attached or absent altogether [36]. This lack of structural support leads to leaky vessels and therefore inconsistent blood flow, which may impede lymphocyte trafficking [35]. Given that irregular blood flow inhibits lymphocyte trafficking, increased blood flow would seem favourable to tumour infiltration by effector cells. However, overexpression of substances that promote angiogenesis in solid tumours, such as vascular endothelial growth factor (VEGF), can result in reduced trafficking [37]. When VEGF is produced in high levels, adhesion molecules are downregulated [37]. These molecules, such as intercellular cell-adhesion molecule-1, play a role in transmigration of the T-cell across the wall of the blood vessel into the tumour site [38]. As such, when their expression is suppressed, T-cell trafficking is reduced despite increased blood flow [39]. To circumvent the issue of trafficking, injection of CAR T-cells into the tumour itself or the surrounding region has been considered. Three clinical trials are currently active that are exploring the use of regional injection to treat mesothelioma and ovarian, lung, breast, and head and neck cancers and are projected to conclude in 2019 [40, 41, 42].

Even if CAR T-cells successfully make their way into the solid tumour, the tumour microenvironment (TME) itself generates conditions unfavourable to antitumour activity. CAR T-cells in the TME face hypoxia, which promotes glycolysis and therefore the production of lactic acid [28]. In a sufficiently acidic environment, the proliferation of T-cells and the production of cytokines that promote it is reduced [43]. Immunosuppressive soluble factors in the TME also pose a challenge. For instance, prostaglandin E2 is produced by tumour cells, and impedes T-cells' ability to proliferate by suppressing their expression of IL-2 [44]. Fourth-generation CAR T-cells provide a tailored approach to solid tumours by addressing the reduced expression of cytokines in the TME that are favourable to anti-tumour activity [27]. Physically, these specialized CAR T-cell are comprised of an ordinary CAR T-cell along with an expression cassette that is reactive to nuclear factor of activated T-cell (NFAT) proteins [45]. An expression cassette is a portion of vector DNA that includes a gene and a promoter [46]. Union of the T-cell's CAR with the target antigen results in the activation of the NFAT-responsive promoter, and the corresponding gene on the expression cassette is then transcribed [45]. This gene typically codes for a cytokine or chemokine that promotes inflammation such as interleukin-12 (IL-12), and when deposited in the targeted tissue by the CAR T-cell, can recruit additional immune cells to the site [45]. This concept is especially helpful with respect to the phenotypic heterogeneity of the cells found in solid tumours: by attracting an assortment of immune cells, even cells not expressing the relevant TAA can be targeted [45]. IL-12 in particular has also been shown to increase the cytotoxicity of T-cells themselves [47]. In general, the therapeutic use of IL-12 is limited by the toxicity that accompanies its systemic administration, thus making fourth-generation CAR T-cells an appealing mechanism for local deposition of IL-12 and other cytokines into the tumour itself [48].

5. Toxicity

When it comes to the clinical application of CAR T-cells, the toxicity that accompanies their administration is a primary concern. A variety of toxicities due to CAR T-cell treatment have been documented, with cytokine release syndrome (CRS) being the most common [49, 50]. CRS is caused by an increase in serum cytokines, such as interferon-γ and interleukin-6 (IL-6), that are released by CAR T-cells following their activation upon engagement with an antigen [51]. Symptoms are varied and can include fever exceeding 40.0°C, nausea, vomiting, mental status changes, and seizures [52]. The severity of CRS can range from mild to severe and is defined on a scale of 1 to 5 [52]. Grade 1 CRS requires only symptomatic treatment, such as antiemetics or medications to reduce fever [52]. CRS is defined as Grade 4 when symptoms become life-threatening and the patient requires more extensive medical assistance, such as use of a ventilator [52]. Grade 5 CRS results in death [52]. The severity of CRS correlates with extent of the patient's disease at the time of infusion, with a higher disease burden corresponding to more severe CRS [53]. In a Phase 1-2a study of tisagenlecleucel for the treatment of relapsed or refractory ALL, CRS occurred in 88% of patients [54]. Severe CRS, defined as CRS requiring respiratory or hemodynamic support, occurred in 27% of patients [54]. A Phase 1 trial of axicabtagene ciloleucel demonstrated a similarly high incidence of CRS. 93% of patients experienced CRS, with 13% of patients experiencing Grade 3 or higher, including 1% of patients whose CRS was fatal [3]. In both cases, tocilizumab, an IL-6 receptor antagonist, was used to manage the syndrome [3, 54]. IL-6 is a cytokine that promotes inflammation and of which high levels correlate with severe CRS [55]. Tocilizumab reduces signalling by binding to either soluble or membrane bound IL-6 and preventing its engagement with the corresponding receptors, thereby reversing symptoms of even life-threatening CRS [56]. CAR-T-cell-related encephalopathy syndrome (CRES), also described more generally as neurological toxicity, includes symptoms such as delirium, seizures, inability to speak, muscle spasms, and confusion [51]. Although the cause or causes of CRES are not yet clear, it has been suggested that elevated cytokine levels or direct effects of CAR T-cells on the central nervous system may play a role [57]. On-target off-tumour toxicity is another concern, especially with regards to the identification of new TAAs for attack by CAR T-cells. As mentioned previously, the ideal TAA is expressed consistently on cancer cells and at low levels, if at all, on normal tissues [28]. On-target off-tumour toxicity occurs when CAR T-cells attack the aforementioned normal tissues that express the relevant TAA, which has resulted in symptoms ranging from B-cell depletion to death [51]. In a study examining the efficacy of HER2-targeting CAR T-cells for the treatment of solid tumours, a patient with metastatic colon cancer developed respiratory distress, low blood pressure, decreased heart rate, and gastrointestinal bleeding, leading to her death [58]. It has been suggested that this fatality was due to the high magnitude of the dosage of CAR T-cells, which engaged with HER2 molecules found in pulmonary tissue, subsequently releasing inflammatory cytokines that led to severe CRS and death [58]. Further research using HER2-targeting CAR T-cells at a lower dose has shown underwhelming results, with only four out of seventeen patients showing stable disease following treatment [59]. However, these trials have also shown minimal toxicity, with only one patient experiencing a fever that was alleviated with ibuprofen [59].

6. Avenues for Future Research

Due to the recent nature of the literature surrounding CAR T-cells, a great deal of further research is needed. The theoretical long-term effects have not yet been observed. Certain blood cancers have demonstrated potential resistance to CAR T-cells via lineage switches, a process in which the phenotype of cancer cells changes [60]. In the case of leukemia, treatment with CD19-targeting CAR T-cells can cause antigen loss, and this plasticity may render CAR T-cells ineffective with respect to long-term immunosurveillance [60]. In addition, the use of lentiviruses as a vector may pose risks to the patient should vector DNA be inserted into the T-cell, potentially causing the T-cell to become malignant in a process known as insertional oncogenesis [61]. The locus at which the majority of insertional oncogenesis takes place is silent in T-cells, thus making the risk of mutagenesis low, however future research is warranted [57]. The management of toxicity is also an area of high importance. Suicide genes, or genes that allow for the destruction of CAR T-cells within the patient's body should toxicity occur upon infusion, have been proposed [57]. For example, the herpes simplex thymidine kinase allows modified T-cells to be killed by Ganciclovir, an antiviral medication [62]. However, because the herpes simplex thymidine kinase tends to provoke an immune response, these cells may be rejected [57]. Another avenue that would make targeted CAR T-cell death possible is the expression of an antigen by the T-cell for which there is a corresponding monoclonal antibody [63]. For example, a CAR T-cell expressing CD20 could be destroyed by rituximab [63].

7. Conclusion

Ultimately, a balancing act on the part of researchers is what is needed to make CAR T-cells applicable to a wide variety of clinical situations. Mitigating toxicity while increasing the potency of CAR T-cells, with respect to solid tumours in particular, will widen their use beyond just blood cancers that express CD19.

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