The safety and efficacy of CAR-T cells in the treatment of prostate cancer: review

Othman Mohammad Saleh, Khaled Anwer Albakri, Yasmeen Jamal Alabdallat, Majd Hamdi Dajani & Walaa Bayoumie El Gazzar

To cite this article: Othman Mohammad Saleh, Khaled Anwer Albakri, Yasmeen Jamal Alabdallat, Majd Hamdi Dajani & Walaa Bayoumie El Gazzar (2021): The safety and efficacy of CAR-T cells in the treatment of prostate cancer: review, Biomarkers, DOI: 10.1080/1354750X.2021.2016973

To link to this article: https://doi.org/10.1080/1354750X.2021.2016973

Published online: 20 Dec 2021.

Article views: 40
The safety and efficacy of CAR-T cells in the treatment of prostate cancer: review

Othman Mohammad Saleha, Khaled Anwer Albakria, Yasmeen Jamal Alabdallat, Majd Hamdi Dajania and Walaa Bayoumie El Gazzarb,c

aMedical Student, Faculty of Medicine, Hashemite University, Zarqa, Jordan; bDepartment of Basic medical sciences, Faculty of Medicine, Hashemite University, Zarqa, Jordan; cDepartment of Medical Biochemistry and molecular biology, Faculty of Medicine, Benha University, Benha city, Egypt

ABSTRACT

Objective: A new breakthrough development in cancer treatment is chimeric antigen receptor (CAR)-T cell therapy. In this review, we focussed on its efficacy & safety in prostate cancer, obstacles impeding its clinical use, and some strategies trying to overcome them.

Methods: Searching for relevant articles was done using the PubMed and Cochrane Library databases. Studies had to be published in full-text in English in order to be considered.

Results: Many factors can limit optimal CAR-T cell outcomes, including the hostile Prostate microenvironment, age, comorbidities, and tumour grade. The adverse effects of the therapy, particularly the cytokine release syndrome, are a major source of worry after treatment administration. Attempts to alter gamma/delta T-cells and NK cells with CAR, on the other hand, have demonstrated higher effectiveness and safety than conventional CAR-T cells.

Conclusion: To improve the use of immunotherapies, a greater understanding of the prostate cancer microenvironment is required. Concerning toxicity, more research is needed to find the most specific and highly expressed prostate antigens. Furthermore, discovering predictive biomarkers for toxicities, as well as choosing the correct patient for therapy, might decrease immune-related side effects and achieve a greater response.

Abbreviations: PCa: Prostate Cancer; GCO: Global Cancer Observatory; CAR: Chimeric Antigen Receptor; PSA: Prostate Specific Antigen; mpMRI: multi-parametric magnetic resonance imaging; PCA3: Prostate CancerAntigen3); PHI: The Prostate Health Index; TMI: Tumour Microenvironment; CTLs: Cytotoxic T Lymphocytes; TAMs: Tumour-Associated Macrophages; MDSCs: Myeloid-Derived Suppressor Cells; CAFs: Cancer-Associated Fibroblasts; ROS: Reactive Oxygen Species; TGF-β: Transforming Growth Factor; scFv: single-chain variable fragment; TM: Transmembrane Domain; NK: Natural Killer; IFN-γ: Interferon-gamma; TNFα: tumour necrosis factor-α; TCR: T cell receptor; MHC: Major Histocompatibility Complex; TRUCKs: T cells redirected for antigen-unrestricted cytokine-initiated killing; JAKs: Janus kinases; STAT: signal transducer and activator of transcription; VEGF: Vascular endothelial growth factor; TAA: Tumour-Associated Antigens; PAP: prostatic acid phosphatase; PSA: prostate-specific antigen; PSMA: prostate-specific membrane antigen; PSCA: prostate stem cell antigen; ADT: Androgen deprivation therapy; GPI: Glycosylphosphatidylinositol; EpCAM: Epithelial cell adhesion molecules; DCs: Dendritic cells; CRS: cytokine release syndrome; PCR: polymerase chain reaction

Introduction

Prostate cancer (PCa) is the second most prevalent cancer and the sixth deadliest cancer among men (Handa et al. 2020, John 2021). According to the Global Cancer Observatory (GCO), it is estimated that the incidence of prostate cancer is approximately one million and a half worldwide, especially in Europe and Asia, and the number of deaths is 375,304 in 2020 (Globocan, 2020, John 2021). Although the mortality rate is low compared to other types of cancer, patients with advanced prostate cancer may develop a more resistant type of cancer called castrate-resistant prostate cancer that does not respond to hormonal therapies (Shaffer and Scher 2003, Bansal et al. 2021). While Surgery and radiation therapy showed successful eradication of early tumours, the traditional treatments are not effective (Litwin and Tan 2017).

Cancer immunotherapy focussed on T cells has now revolutionised the field of oncology through different approaches such as immune checkpoint blockade, adoptive cellular therapies, and cancer vaccines.

Immune checkpoint blockade has proven to be an effective approach in the treatment of a broad spectrum of cancers over the last decade. It is designed to interfere with the T cell inhibitory pathways and to enhance the function of antitumor T lymphocytes (Hargadon et al. 2018). However, there are several challenges still being faced regarding immune checkpoint blockade therapy in prostate cancer as it is considered an immunologically cold tumour with minimal T cell infiltrates.
and very limited response to single-agent checkpoint inhibition (Topalian et al. 2012, Kwon et al. 2014, Hansen et al. 2018). Thus, the development of genetically engineered T cells directed against cancer antigens would represent a solution to overcome this immunological tolerance.

The chimeric antigen receptor CAR-T cell therapy has caught more attention in applying it on solid tumours, and great efforts are made to enhance the design of CAR T cells for longer survival in vivo and to be more robust against solid tumours (Thakur et al. 2020). This new therapy takes advantage of the unique antigenic representation on the prostate cancer cells by infusing ex-vivo re-engineered host immune T cells to target those specific antigens (Hillerdal and Essand 2015, Wang and Riviere 2016).

In this review, we aimed to shed light on the role of T cells in cancer immunotherapy, focussing on the recent progress in the chimeric antigen receptor (CAR)-T cell therapy in PCa, emerging targets, challenges that hinder its clinical application, and suggest strategies to overcome these challenges & further improve treatment efficacy.

Overview of prostate cancer
Prostate cancer develops from mutations in prostate glandular cells and most often starts from the peripheral basal cells (Lee and Shen 2015). Prostate cancer is mostly presented in two broad categories: localized early cancer and castration-resistant metastatic cancer. In the localized form, the most common genetic mutation is the translocations of the ETS genes, while in the castration-resistant form, the most common genetic mutations affect androgen receptor and onco-suppressors TP53 and PTEN (Mansinho et al. 2018). The chances to develop prostate cancer get higher as the age gets older, with an incidence peak between 75 and 79 years older men in the UK (Cancer Research UK 2017). Ethnicity also plays a significant role as a risk factor, African American and South American men have the highest rates of incidence and mortality compared to white men; this might be linked to chromosome 8q24 variations, cell apoptosis genes like BCL2 variation, and tumor suppressor genes such as EphB2 variation, that are shown in African American populations (Whitman et al. 2010, Jayadevappa et al. 2011, Wu and Modlin 2012). Other risk factors have been reported including: family history, insulin-like growth receptors, sexually transmitted diseases, and lifestyle (i.e., smoking, obesity, alcohol consumption) (Perdana et al. 2016).

Regarding the latest outlines, a prostate cancer diagnosis is carried out through multiple steps, starting with prostate-specific antigen (PSA) testing; elevated PSA level higher than 4ng/ml indicates an increased risk of prostate cancer and justifies further investigation. In addition, 40 and 60 years old individuals with PSA levels higher than 1ng/ml and 2ng/ml, respectively, indicate an increased risk for prostate cancer metastasis (Cuzick et al. 2014, Parker et al. 2020). If the PSA levels appeared to be elevated, multi-parametric magnetic resonance imaging (mpMRI) would be the next step before biopsy (Rouviere et al. 2019, Parker et al. 2020). If the cancer was doubted, a biopsy should be taken using ultrasound guidance which is the most common technique (Verma et al. 2017). Although PSA testing is effective as a wide range of screening tools but using it is still controversial, and other alternatives are available like Prostate Cancer Antigen 3 (PCA3) and The Prostate Health Index (PHI) (Narayan 2020).

Prostate cancer microenvironment
The tumour microenvironment (TME) is the distinctive landscape surrounding the tumour. It has a significant role in the development of cancer as well as the response to immunotherapy. Multiple immune cell types are present in the PCA microenvironment; of these immune cells, T cells, particularly cytotoxic T lymphocytes (CTLs), are most obviously vital during immune-mediated clearance of tumours (Allen et al. 2015). In addition, aggressive PCa has been linked with the presence of B cells (Woo et al. 2014), tumour-associated macrophages (TAMs), tumour-associated neutrophils, myeloid-derived suppressor cells (MDSCs) (Hui and Chen 2015). Although Cancer-associated fibroblasts (CAFs) are not immune cells, they are major players in the immunosuppressive circuitry of tumours (Shalapour et al. 2015).

The normal intra-tumoral microenvironment of PCa is characterised by low T cell density, particularly in the CD8+ fraction. This exclusion of T cells from the TME contributes to the formation of an immune-privileged area (Philippou et al. 2020, Wu et al. 2020). Hypoxic cores, which are commonly present in PCa, lack T cell infiltration and induce aberrant angiogenesis programs that promote neovascularization capable of inhibiting CTL extravesation. (Motz et al. 2014). Therefore, even after obtaining access to the tumour, T cells are usually met by a highly suppressive PCA TME (Shalapour et al. 2015, Bezzi et al. 2018, Jayaprakash et al. 2018).

One of the substances that can be metabolised by cancer is lactic acid, a product of glycolysis under low oxygen conditions. However, even when oxygen levels are normal, cancer cells choose to go through glycolysis and lactate metabolism (Romero-Garcia et al. 2016). Choi et al. proposed that a high concentration of lactic acid in the TME, which increases its acidity, is a new immunological escape mechanism that suppresses immune cells in TME (Choi et al. 2013). The tumour can accomplish that suppression through disrupting the normal functions of leukocytes as follows; Natural killer cells: reduces their ability to secrete perforin and granzymes; Neutrophils: declines reactive oxygen species (ROS) secretion and poor phagocytosis; Cytotoxic T lymphocytes: decreases their recruitment and inhibit the infiltrated ones; Regulatory T cells: impedes their activity and recruitment (Wang et al. 2020).

Myeloid-derived suppressor cells are another strong determinant of TME immunosuppression. It can generate chemicals that eventually downregulate the T cell receptor (Baniyash 2004), resulting in T cell anergy, as well as release ROS that triggers T cell apoptosis (Fleming et al. 2018). In addition to IL-10 and transforming growth factor (TGF) generated by MDSC, it can promote CD4+ T cell development into regulatory T cells, both of which are able to inhibit immune systems (Munn et al. 2005, Trikha and Carson 2014). A study has shown that prostate cancer cells produce substances that cause monocytes and macrophages to flock to the area (Lo and Lynch 2018). After being recruited into the microenvironment, macrophages are exposed to a variety of environmental signals that might cause them to polarise into pro-or anti-inflammatory states (M1 or M2), respectively (Pollard 2004). M2 infiltration has been observed to correspond with disease aggression, poor prostate cancer prognosis (Lin et al. 2016), and inhibit T cell growth (Huber et al. 2010).
A better understanding of the PCa microenvironment, particularly the molecular basis and behaviour of the immune cells resident within these niches, would enhance the use of immunotherapies and provide the means for bypassing immune tolerance mechanisms.

**Before infusing CAR-T cell**

Prior to administering CAR T cells, a lymphodepleting regimen is performed to boost the efficiency and durability of CAR T cells. It acts in a variety of ways, such as removing cytokine sinks (IL-2, IL-7, and IL-15) and eliminating immune-suppressive cells (regulatory T cells and myeloid-derived suppressor cells). It can also boost the growth and survival of CAR T cells (Gattinoni et al. 2005, Ninomiya et al. 2015).

Ramos et al. demonstrated that anti-CD30 CAR-T Cell therapy administered with a fludarabine-containing regimen to Hodgkin Lymphoma patients resulted in more strong clinical responses than using bendamustine alone (Ramos et al. 2020). Also, Administering of fludarabine plus cyclophosphamide (Cy/Flu), a lymphodepleting regimen, was associated with increase of anti-CD19 CAR-T cell proliferation and persistence in non-Hodgkin lymphoma patients (Turtle et al. 2016). A pre-clinical trial on mice showed that infusing PSCA-CAR T cells with cyclophosphamide to target PSA-cancer bone metastases and pancreatic metastases resulted in good anti-tumour responses (Neelapu 2019). So, using the appropriate lymphodepletion regimen, with taking toxicity into consideration, seems to achieve better results than not using it.

**Structure and mechanism of action of CAR-T cells**

CAR T-cell is a biological therapy that uses specific modified T cells to attack malignant tissues by targeting antigens on their surface (Chavez et al. 2019). CARs are artificial receptors consisting of a binding domain which is an extracellular single-chain variable fragment (scFv) that specifically recognises a tumour-associated antigen, transmembrane domain (TM), and endodomain (Yu et al. 2017). The extracellular domain (scFv) is derived from antibodies that can target displayed antigens on the surface of the malignant cell (Maher et al. 2016). TM acts as a link between the extracellular domain and the intracellular domain and anchors the CARs to the T-cell plasma membrane (Chandran and Klebanoff 2019). The endodomain is the active part, which is responsible for activating and sending the signal to the T cell, which ultimately promotes CAR T-cell stimulation and expansion (Tasian and Gardner 2015).

Some instruments are used to pick specific subgroups of T cells such as CD4+ and CD8+ from the host, which then pass through sequential stages to end up with CAR T-cell (Wang and Rivière 2016). T cells start proliferating and secreting many cytokines after the connection between the specific tumour antigen and scFv occurs (Zhang et al. 2020). These cytokines stimulate other immune cells to produce cytokines (Shimabukuro-Vornhagen et al. 2018). CD4+ cells mediate antitumor immunity by provoking CD8+ T cells, natural killer (NK) cells, and other innate immune cell types (van der Leun et al. 2020). CD8+ and CD4+ cells have a direct effect against tumour cells by excreting Interferon-gamma (IFN-γ) and tumour necrosis factor-α (TNFα), which ultimately lead to cell death (Tay et al. 2021). They also release perforin and granzymes, which bring about apoptosis. (Yasukawa et al. 2000). So, the killing mechanism is similar to the way that human body T cells work, which involves binding of the T cell receptor (TCR) to the presented antigen on a major histocompatibility complex (MHC) molecule and then the signal start (Smith-Garvin et al. 2009). The prominent feature of CAR T cell is that it does not need the antigen to be presented to recognise it; the intact proteins are enough (Eshhar et al. 1993). This is useful when MHC expression is downregulated on cancer cells (Garrido et al. 2016).

Human T cell efficacy and persistence had reduced because of the harsh cancer microenvironment. So, the CAR T cell emerged trying to solve some of the T cell problems, and each generation helped engineer the next one. Five generations of CAR T-cell have been produced with different endodomain compositions (Figure 1). The first generation consists only of CD3ζ as endodomain, but it lacked a costimulatory molecule and showed insufficient proliferation and persistence inside the body (Sha et al. 2017). So, to expand the cells more and make them stay for a longer period, to secrete extra cytokines, the second generation appeared, which consists of CD3ζ with the addition of a costimulatory signalling domain (CD28 or 41BB) (Pang et al. 2018). Incorporating the costimulatory CD28 and OX40 together or CD28 and 41BB together, known as the third generation, showed a better clinical response and more resistance of the microenvironment than the second generation (Hombach et al. 2013, Whilding and Maher 2015). To overcome the heterogeneity in solid tumours and induce the innate immunity against cancers-resistant conventional CAR T cell, further modifications on CAR T-cell led to the emergence of a new generation called the fourth generation of CAR T-cell or TRUCKs (‘T cells redirected for antigen-unrestricted cytokine-initiated killing’) (Chmielewski and Abken 2015). It is based on the second generation structure, but additionally modified with a constitutive or inducible expression cassette for a transgenic protein, such as interleukin IL-12 cytokine, which is released by the CAR T cell to modulate the T-cell response. IL-12 improves T-cell activation, modulates the immunological and vascular tumour environment, and recruits additional immune cells to fight cancer cells that are not recognised by CAR T cells (Chmielewski and Abken 2015), and improves the expansion and persistence of CAR T cell against tumour environment (Koneru et al. 2015).

Another structure of TRUCKs is constructed to secrete different types of molecules such as: IL-18, IL-15, or IL-7, and they were less toxic than IL-12 (Krenciute et al. 2017, Shum et al. 2017, Avanzi et al. 2018). Now a new generation of CAR (fifth generation) is being tested. Its structure is also based on the second generation with a fragment of IL-2 receptor β. This fragment can induce the mRNA expression of Janus kinases (JAKs) and signal transducer and activator of
transcription (STAT)-3/5. The binding of antigen with this receptor can trigger all three cooperative signals (through the CD3ζ domains, CD28 domain, and cytokine (JAK–STAT3/5)), which provides full T cell activation and proliferation (Tokarew et al. 2019, Qu et al. 2020).

CAR T-cell therapy has shown favourable results in treating patients with Non-Hodgkin’s lymphoma, Acute lymphoblastic leukaemia, and Chronic lymphocytic leukaemia (Maude and Barrett 2016, Pehlivian et al. 2018, Kersten et al. 2020). However, its effect against solid tumours such as sarcoma, breast cancer, and prostate cancer was disappointing (Junghans et al. 2016; Tchou et al. 2017). This could be related to the polyclonality and the immunosuppressive pattern of solid tumours microenvironments.

**Challenges and potential strategies for an effective prostate cancer CAR-T cell therapy**

It was believed that the increase in tumour angiogenesis via proangiogenic factors (VEGF) could provide more vessels for possible CAR T cell trafficking, but counter-intuitively, the tumour vasculature is structurally and functionally abnormal. This abnormality enhances the progression of the tumour by reducing perfusion resulting in hypoxia and low pH level, which also limits the immune cells entry into tumours (Jain 2014). Pharmacological inhibition of VEGF signalling enhanced T-lymphocyte recruitment and supported the use of antiangiogenic agents to increase the efficacy of cancer immunotherapy (Huang et al. 2015). Administrating antiangiogenic agents, in doses that normalise the vasculature, alongside the immunotherapy have improved the response significantly (Huang et al. 2012, Shi et al. 2013).

Chemokines can recruit different immune cells that have various functions in tumour regression (Nagarsheth et al. 2017). Chemokine receptor CXCR3 is highly expressed on effector T cells, helper T cells, and natural killer cells, which all have anti-tumoral effects. The Chemokine ligands CXCL9 and CXCL10, which bind to CXCR3, are potent T cell attractants and lead to a pro-inflammatory response (Zhang et al. 2019). As a result, some researchers investigated the possibility of attracting more CAR T cells to tumour cells by integrating a chemokine receptor gene with CAR-T cells (Hill et al. 2018, Abu-5beih et al. 2019) whereas others have looked towards designing CAR-T cells that able to produce several chemokines on their own (Blumenberg et al. 2020).

Otherwise, immunosuppressive chemokines that are secreted by tumours can boost the growth rate of a tumour and its metastasis. This challenge could be overcome by combination therapies, such as giving CAR-T cells with an immune checkpoint inhibitor or with androgen-deprivation therapy, which showed high T-cells activation and high T cells proliferation, respectively (John et al. 2013, Sanchez et al. 2013).

Tumour resistance to a single antigen that is targeted by CAR constructs is one of the most challenging constraints of CAR-T cell therapy. This could be explained by the tumour plasticity and heterogeneity which lead to loss of the expression of target antigens in malignant cells, either partially or completely (Watanabe et al. 2018, Sterner and Sterner 2021). This kind of resistance has been found in solid tumours (O’Rourke et al. 2017). However, targeting several antigens is a solution to this problem. Up to now, there are several available essential multi-antigen-targeted CAR-T cell treatments, including Pooled CAR-Ts cells that are made up of two modified T cell lines, each of which expresses a distinct
antigen recognised by CAR; Dual CAR-T cells have two CARs, each of which possesses a full signal domain that initiates CAR-T cells’ antitumor activity; Tandem CAR-T cells can activate T cells when one CAR is connected to two separate antigen-binding domains in tandem; Trivalent CAR T cells have three CARs presented on a single modified T cell and target approved antigens (Han et al. 2019).

A major obstacle regarding CAR T cell clinical efficacy is its poor in vivo persistence (Lamers et al. 2005, Kershaw et al. 2006, Jensen et al. 2010). Therefore enhancing CAR T cells survival has been the main concern of many recent studies (Chmielewski and Abken 2015; Guedan et al. 2018, Qu et al. 2020). Usually, the effectiveness of CAR T cells is investigated in bulk CD4+ and CD8+ without taking into account the independent contribution of each subtype (Abe et al. 1995). However, Guedan et al. have discovered that CD4+ CAR T cells with ICOS (Inducible T-cell COStimulator) intracellular domain had a synergistic impact when combined with CD8+ CAR T cells expressing 4-1BB intracellular domain. Thus, a balance between CD4 and CD8 cells is important to have a high persistence and activity (Guedan et al. 2018).

Prostate tumour antigens

The initial and most important step to achieve the utmost effective CAR-T cell therapy is to identify prostate TAA (Tumour-Associated Antigens). Targeting a tumour-restricted antigen should be considered to avoid immune reactions against healthy tissues (Hinrichs and Restifo 2013, Mirzaei et al. 2017). Prostate tumours contain several tumour-specific antigens like prostatic acid phosphatase (PAP), prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA), and although these have limited antigenicity, their high specificity makes these antigens prime candidates for immunotherapies (Correale et al. 1997, Knight et al. 2009, Olson et al. 2010). Therefore, many clinical trials have recently concentrated on the use of TAAAs as an activation method to cause an immunological reaction in prostate cancer patients and showed promising findings stated that this strategy is safe and feasible (Kiiessling et al. 2012, Westdorp et al. 2014).

Prostate-specific membrane antigen (PSMA)

PSMA is found on the prostate cancer cells, thus considered an appropriate candidate for antigen-redirected immunotherapy (Mesters et al. 2006). These antigens are presented in all tumour phases, and they can display an elevated expression in androgen-independent and metastatic stages of the disease (Wright et al. 1996, Kawakami and Nakayama, 1997, Silver et al. 1997). Regarding diagnostic purposes and for the development of antibody-based treatments, numerous protective proteins produced by the immune system have been designed to target PSMA (Slovin 2005).

Anti-PSMA CAR-T cells have been previously proven to be targeting tumours (Maher et al. 2002, Gade et al. 2005). Furthermore, it has been demonstrated a significant justification for the continuing assessment of PSMA-targeting approaches for the treatment of prostate cancer. Zuccolotto et al. studied PSMA CAR-T cell molecular interactions and reported that it efficiently eliminated diffused tumour cells after in-vivo transfer in tumour-bearing mice (Zuccolotto et al. 2014).

Phase I clinical trial has been conducted by Slovin et al. to study the clinical effectiveness of administering anti-PSMA CAR-T cell therapy. They demonstrated that the clinical symptoms of patients who received a certain dose of anti-PSMA CAR T cell have stable disease and stable scans while the patients who received a larger dose had a moderate fever and high levels of IL-4, IL-8, IL-6, and others. Thus, we can treat patients with PSMA targeted T cells as a safe drug by using a certain dose (Slovin et al. 2013).

Several studies have been done to assess the efficacy of the anti-PSMA CAR T cell in combination with chemotherapeutic agents, vaccines, immune checkpoint inhibitors, and cytokines like IL-23, TGF-ß, and IL-2. The clinical usage of IL-2 with CAR T cell is still controversial. According to some studies, IL-2 is essential for CAR-T to eradicate solid tumours in animal models (Moeller et al. 2005, Lo et al. 2010). However, a study conducted by Junghans et al. 2016 found no anti-tumour activity either with strong in vivo expansions and low infused IL2 levels (Junghans et al. 2016) . Despite the fact that IL-2 promotes T-cell proliferation and enhances effector T-cell activity, it also has an unanticipated immunosuppressive role by binding to CD25-expressing naïve CD4+ T cells, leading to their differentiation and maturation into CD4+ Tregs (Ye et al. 2018). So, this could explain the heterogeneity of the previous studies results.

TGF-ß is one of the cytokines that exist in a high concentration in serum and tumours prostate cancer patients (Shariat et al. 2004). It has a dual function as it suppresses the growth of the tumour by inhibiting its proliferation and stimulating apoptosis in the early stage of cancer, while in advanced stages, it promotes its progression to metastasis (Zhu and Kyriianou 2008). TGF-ß induces a signal through binding with TGF-ß receptor that consists of tetrameric structure (two of type I TGF-ß receptors (TGF-ßRI) and two of type II TGF-ß receptors (TGF-ßRII) (Wrana et al. 1994). If we remove the kinase domain of TGF-ßRII, we will have a dominant-negative form of TGF-ßRII, and previous studies demonstrated that by using it, we could block the TGF-ß inhibitory signalling (Gorelik and Flavell 2000). A phase I clinical trial is now conducting by Kloss et al. (NCT03089203) to test the effectiveness of anti-PSMA CAR with co-expressing of dominant-negative TGF-ßRII in T cell (Kloss et al. 2018).

One of the inflammatory cytokines that is found to play a role in the formation of tumours is interleukin-23 (IL-23). Besides promoting the tumour inflammatory response via angiogenesis and infiltrations of M2 macrophages and neutrophils, it can reduce the leakage of CD4+ T cells and CD8+ T cells into the tumour tissue (Nie et al. 2017). Therefore, in a recent study conducted by Wang et al. in 2020, they designed a new CAR structure called IL23mAbT2APSMA-CAR T cells and concluded that IL-23mAb
combined PSMA CARs were more effective than PSMA CAR only in Prostate Cancer Eradication (Wang et al. 2020).

**Prostate-specific antigen (PSA)**

Several studies provide evidence about the feasibility of using vaccines targeting PSA as a therapy for prostate cancer, as it was found that PSA could cause a particular T cell response (Sanda et al. 1999, Noguchi et al. 2003). However, up to our knowledge, there are no clinical trials that discussed the efficacy of using PSA as a target to attack tumour cells among human beings. This could be because PSA is classified as a self-antigen and shows a similar structure to kallikrein, which leads to immune tolerance. Consequently, we should use combination therapy to overcome this challenge. Arredouani et al. examined the impact of androgen deprivation therapy (ADT) on T lymphocytes among transgenic mice and showed that there is an obvious increase in PSA-specific cytotoxic lymphocytes (Arredouani et al. 2010). On the other hand, ADT is not useful for patients with castrate-resistant prostate cancer. Therefore, future research should focus on finding high specific receptors corresponding with PSA, which may be done by engineering new CAR molecules.

**Prostate stem cell antigen (PSCA)**

PSCA is a glycosylphosphatidylinositol (GPI)-anchored protein encoded from a gene located on chromosome 8q24.2. It is expressed mainly in epithelial cells of the prostate, kidney, and small intestine. Reiter et al. found that PSCA was highly presented in prostate cancer (Reiter et al. 1998). It signals the shift of prostate epithelial cells from a highly proliferative to a more differentiated state, and it may be used to identify a subpopulation of cells that are vulnerable to transformation during prostate carcinogenesis (Uzgare et al. 2004).

Over the past decade, PSCA-specific CARs showed promising findings against prostate cancer. Kloss et al. reported that co-transduced T cells destroy tumours that express both PSMA and PSCA (Kloss et al. 2013). Furthermore, a study conducted by Priceman et al. revealed that giving PSCA-specific CARs, which contain 4-1BB, a co-stimulatory signalling domain, to mice with prostate cancer has a strong therapeutic efficacy (Priceman et al. 2018).

**Epithelial cell adhesion molecules (EpCAM)**

EpCAM, also called CD326, is an antigen expressed by prostate cancer and other solid tumours (Ni et al. 2012). It was found that EpCAM-CAR T cells could kill prostate cancer cells with increased EpCAM expression and prolong the survival in low-expressing cells (Deng et al. 2015). However, using this molecule against metastatic prostate cancer should be investigated.

**Types of immunological cells that correspond with CAR molecule**

**CAR gamma Delta-T cells**

Gamma delta (γδ) T cells, a type of T-lymphocyte, have a TCR composed of one (gamma) chain and one (delta) chain. It’s less prevalent than αβ T cells, but it’s found in greater numbers in the gut mucosa, lungs, and the reproductive tract (Holtmeier and Kabelitz 2005, Bonneville et al. 2010).

A significant difference exists between γδ T cells and αβ T cells in terms of their ability to bind to antigens, αβ T cells cannot bind to antigen directly and must rely on the presence of the major histocompatibility complex (MHC)-restricted. On the other hand, the underlying principle of γδ T cells-antigen binding is dependent on the intact antigen’s conformational shape, and it can detect a variety of antigens without the involvement of MHC as they do not have CD4 or CD8 to self-recognise MHC molecules (Chapman et al. 2015). This feature opens up the possibility of developing immunotherapies for cancers without well-defined neoantigens and without the need for additional genetic engineering.

Tumour cells become resistant to αβ CAR T-cell mediated cytotoxicity or generate anergy in particular T cells when the expressed antigens are lost, MHC molecules are reduced, and costimulatory molecules are missing. Mirzaei et al. said that: ‘We postulate that several characteristics of γδ T cells make them an attractive T cell subset to apply CAR T cell therapy for solid tumours, including their inherent antitumor activity and ability to home to epithelial tissues’ (Mirzaei et al. 2016).

Liu and colleagues reported that treatment with syngeneic γδ T cells showed marked antitumor efficacy in prostate tumour-bearing mice (Liu et al. 2008). In another study by Liu et al. (2005), the ex vivo amplified Vγ9Vδ2 γδ T cells that resist apoptosis were able to intrinsically detect and destroy human prostate tumour cell lines in vitro.

γδ T cells also have an indirect antitumor activity as they also facilitate the function of other immune cells, such as dendritic cells (DCs), B cells, and CD8+ T cells (Liu and Zhang 2020).

A recent study suggests that γδ CAR T cells targeting PSCA extend longevity, protect against tumour-based illnesses, and greatly attenuate tumour-induced osteolysis in a mouse model (Abate-Daga et al. 2019)

**Natural killer cells and chimeric antigen receptor therapy (CAR-NK)**

A novel alternative promising treatment is the use of modified NK cells instead of CAR T cells. Natural killer cells can destroy viruses and pathogen-infected cells by secreting perforin, granzymes, and Interferon gamma (Sun and Lanier 2011). Also, it has a physiological ability to kill malignant cells normally. According to recent studies, tumours spread faster in spontaneous leukaemia and prostate cancer models with reduced NK cells than in those with normal NK cell activity (Guerra et al. 2008). Therefore, incorporating CAR into NK cell could enhance and magnify NK cytotoxicity (Wrona et al. 2021).
Table 1. Comparison between CAR T cell and CAR NK cell.

<table>
<thead>
<tr>
<th></th>
<th>CAR T cell</th>
<th>CAR NK cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Released cytokines</td>
<td>IL-1, IL-2, IL-6, IL-10, IL-15, TGF-α</td>
<td>IL-3, IFN-γ, GM-CSF</td>
</tr>
<tr>
<td>Life span</td>
<td>Long</td>
<td>In the absence of cytokines, its persistence is short.</td>
</tr>
<tr>
<td>Capacity of killing</td>
<td>Recognise the targeted antigen on MHC molecule</td>
<td>No requirement for presented antigen to start killing</td>
</tr>
<tr>
<td>Mechanism of killing</td>
<td>By CAR receptor, released cytokines, and activated immune system</td>
<td>By CAR receptor, natural cytotoxicity receptors such as (Nkp30, Nkp44, and Nkp46), and other receptors</td>
</tr>
<tr>
<td>Efficacy on heterogeneous malignant cells</td>
<td>Less effective</td>
<td>More effective</td>
</tr>
<tr>
<td>The risk of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graft versus host disease (GVHD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRS Neurotoxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On-target/off toxicity</td>
<td>High</td>
<td>Low; due to distinct cytokine spectrum release</td>
</tr>
<tr>
<td>Off-the-shelf product</td>
<td>The patient has to wait for the treatment</td>
<td>Accessible on demand</td>
</tr>
</tbody>
</table>

Although CAR T-cell treatments have demonstrated promising results in lymphoid leukemias and lymphomas, as well as myeloma (Maude et al. 2018; Raje et al. 2019), their usage is limited by a number of factors, including cost and side effects such as CRS and neurotoxicity. A recent review showed that CAR-NK cells had been studied preclinically and clinically, and their results suggest an effectiveness and safety profile superior to CAR T cells (Rafei et al. 2021) (Table 1).

NK cells can be obtained from different sources, including umbilical cord and peripheral blood, as well as human embryonic stem cells and immortal NK cell clones. Some of these cell lines are NKG, NKL, and NK-92 cells (Cheng et al. 2013). It has been reported that NK cells are potent mediators of antitumor immunity. For example, one patient with Hodgkin lymphoma and another with multiple myeloma achieved a complete response after NK-92 cell infusion (Williams et al. 2017). However, lack of persistence, absence of cytokine support, and the tumour microenvironment have resisted the therapy and considerably decreased its efficacy (Bi and Tian 2017).

The application of the CAR-NK technique firstly appeared in the context of hematological malignancies treatment then developed to solid tumours (Liu et al. 2021). CAR NK cells targeting CD19 antigen have been developed to attack leukaemia primary CD19-positive cell lines. It has been observed to produce effective CD19-positive B-precursor lysis of leukaemia cell lines (Romanski et al. 2016). In addition, CD5 or CD3 specific CAR-NK92 cells can sustain a stable growth and have potent antitumor activity against T cell leukemia, lymphoma cell lines, and primary malignant cells (Chen et al. 2016, Pinz et al. 2017). In terms of solid tumours, a study suggested that targeting the EGFRvIII (expressed antigen on glioblastoma cells) by CAR-NK-92 cells could kill EGFRvIII-positive glioblastoma cells (Zhang et al. 2016). Also, it showed a high efficacy against ovarian and breast cancers (Klapdor et al. 2019, Liu et al. 2020).

A study conducted by Montagner et al. on mice revealed that recognising PSMA by CAR NK-92 cells exerted a specific cytotoxic activity against positive PSMA cells. Also, they provided a piece of evidence that anti-PSMA CAR-modified NK-92 cells are cost-effective and readily available treatment (Montagner et al. 2020).

Despite CAR-NK advantages, it is still controversial due to the scarcity of clinical trials conducted worldwide, with only 19 ongoing trials listed on clinicaltrials.gov compared to over 500 clinical trials in the subject of CAR-T cell cancer immunotherapy (Wrona et al. 2021). Future in vivo trials is needed.

Memory T cells and chimeric antigen receptor (CAR)

Despite prior modified generations of CAR T cells employing various cell types, CAR T cell growth immediately following injection, as well as long-term persistence after early tumour control, are critical effectiveness determinants (Gattinoni et al. 2017). These characteristics are typical of early-memory T cells e.g., stem cell memory (TSCM) and central memory (TCM) T cells (Arcangeli et al. 2020). Effector memory T cells (TEM) exist in peripheral tissues and mediate rapid effector function (Gattinoni et al. 2017). Although TEM is a memory T cell, its potential for self-renewal is restricted, limiting its utility in therapeutic applications (Gattinoni et al. 2017). Meanwhile TCM and TSCM have a superior role in clinical application due to their highly proliferative capacity (Arcangeli et al. 2020).

There have been two clinical trials in which CD19-specific CAR T cell were produced from isolated TCM (Wang et al. 2016, Gattinoni et al. 2017). In addition, a registered ongoing clinical study (NCT03288493) employing TSCM CAR T cell is currently being evaluated on 220 multiple myeloma patients (ClinicalTrials 2021). As a result, it appears that these cells have a promising future in terms of CAR T cell effectiveness. More clinical studies are needed.

After the administration

To monitor the CAR T cell after administration, a novel approach for detecting the pharmacodynamics of CAR T cells has been developed. CAR T cell is genetically modified to express the herpes simplex virus 1 thymidine kinase (HSV1-tk). Then, it is administered with a 18F radiolabeled 9-[4-18F] fluoro-3-(hydroxymethyl) butyl guanine ([18F] FHBG) as a probe. As a result, we can detect the cells using the PET scan, which gives us an indication of the patient’s prognosis. Surprisingly, no significant adverse effects occurred as a

**Safety considerations**

Despite the positive results that CAR T cell therapy showed against hematological and solid tumours, it causes immune-related adverse effects. Some of them may stay with the patient for several years, and others may ultimately lead to death (Jin et al. 2021). The most life-threatening adverse effect after CAR T cell infusion is cytokine release syndrome (CRS), accompanied by high levels of several cytokines circulating in the blood, especially IL-1 and IL-6 (Lee et al. 2014).

The severity and the risk of CRS are affected by the type of therapy and the characteristics of patients. A phase I clinical trial conducted in 18 males with metastatic castration-resistant prostate cancer revealed severe CRS after administration of PSMA-directed/TGF-β-insensitive CAR-T cells. Administration of this immunotherapy shows a wide range of clinical symptoms associated with CRS include systematic signs (e.g., Fever, skin rash, fatigue, anorexia, myalgias, arthralgias, nausea, vomiting, Tachycardia, capillary leak, and hypotension), other life-threatening symptoms which involve organ dysfunction (e.g., respiratory distress syndrome and renal and/or hepatic failure), and neurologic symptoms (e.g., confusion, delirium, expressive aphasia, and seizures) (Lee et al. 2014, Bonifant et al. 2016).

A study conducted by Abu-Sbeih et al. has shown that one-third of the included patients experienced recurrent diarrhoea, colitis, and bloody stool, which can be caused by changes in microbiome configuration in the gastrointestinal tract. Another CRS-related issue is that the patients are more likely to develop bacterial, viral, and fungal infections in the first 28 days after CAR-T-cell infusion (Hill et al. 2018). So, CAR T-cell transfusion and antibiotics administration can further disrupt the diversity of the gut microbiome contributing to severe adverse effects (Montassier et al. 2015, Blumenberg et al. 2020, Schubert et al. 2021). However, CRS can be managed by providing supportive care, administering anti-IL-6 receptor monoclonal antibodies like tocilizumab and corticosteroids (Maude et al. 2014, Lee and Shen 2015). Hypotension and hypoxia are managed by intravenous fluid and low-dose vasopressors and nasal oxygen supplementation, respectively (Neelapu et al. 2018). IL-1 is one of the cytokines that increase the CRS severity by further recruiting immune cells and inducing tissues to secrete other cytokines (IL-6) and lipid mediators (prostaglandin E2). Thus, a new perspective now is to use CAR T cells with IL-1 receptor inhibitors (Dinarello 2009; Ricciotti and Fitzgerald 2011). Moreover, a wide range of cytokine inhibitors such as the icatibant-selective Janus kinase-1 (JAK-1) inhibitor, can reduce CRS severity (Huarte et al. 2020). JAK is a protein part of Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway that can mediate the release of many cytokines such as IL-6, IL-12, and IFN-γ (Das et al. 2016). So, blocking it would have a beneficial effect of alleviating CRS.

A new generation in the implementation of CAR was proposed by Banerjee et al. 2021 who stated that the use of digital devices and machine learning algorithms might improve CAR T cells’ safety. They demonstrated that if we used wireless continuous temperature monitor devices like (TemTraq or tpatch) and machine learning algorithms, the risk of CAS is likely to be reduced because they will enable clinicians to identify patients who are about to enter CRS and thus deal with it quickly (Banerjee et al. 2021).

The best-targeted antigen is the one that is restricted to the cancerous cell. Infusion of CAR T cells can lead to other types of toxicity such as ‘On-target/off-tumour toxicity’ which means binding of CAR-T cells with the expressed antigen that also exists in the normal cells. It turns out that PSMA, in addition to being found on many types of neoplasms such as the prostate, bladder, and kidneys, but is also presented on many normal cells such as proximal tubules of kidney, liver, small intestine, and colon (Kinoshita et al. 2006). This renders PSMA CAR T-cell as non-specific therapy that can cause autoimmune disease. Therefore, Bispecific T cell and intra-tumoral injection of CAR-T have been developed as safety strategies to minimise On-target/off-tumour toxicity (Yu et al. 2019). In any case, preclinical development of CD126 CAR T-cell, in which multiple types of tumours express CD126, revealed a low risk of CRS (Kloss et al. 2018; Mishra et al. 2021). So, maybe we should start looking for other significant antigens that could be targeted but with fewer adverse effects.

Moreover, regarding the scFv of the most laboratory reorganised T cells come from mice (Zuccolotto et al. 2014; Schepisi et al. 2019), immune rejection occurs due to recognising the infused materials as foreign proteins. As a result, there is a tendency to humanise scFv domain and increase CAR T cell safety and efficacy (Maude et al. 2015).

Another emerging risk that may limit the use of CAR T-cells is the infection of Covid-19. Due to the possibility of false-positive in a polymerase chain reaction (PCR) and nucleic acid test, CAR T-cells is forbidden in a community with a high prevalence of COVID-19, and it must be replaced with an alternative one because if a COVID-19 patient takes CAR T cell therapy, they will suffer from increasing severity of CRS and respiratory distress (Hu et al. 2020, Kanji et al. 2021).

**Criteria of patients who are more suitable to be treated by CAR-T cells**

Although the highly hopeful preclinical findings, clinical trials showed unsatisfactory results. However, it will be highly useful if we find the kind of patients who get maximal benefit with minimal adverse effects. Thus, future studies can focus on these patients and develop new specific modalities to treat them effectively. In any case, because of the few current clinical trials concerning prostate cancer, determining the characteristics of the optimal patients would be based on trials that examined other solid tumours.

According to previous studies that investigate the efficacy and safety of CAR-T cell therapy among solid tumour patients (Wallen et al. 2009, Louis et al. 2011, Ahmed et al. 2015, Katz et al. 2015), young patients showed higher clinical
responses and relatively more favourable safety data than elderly patients. This might be attributed to the elderly’s high prevalence of comorbidities, which reduces their immunity and increases their activated lymphocytes. Despite patients with a low disease burden having a smaller expanded T cells number than those with a high disease burden, they showed higher overall survival and free survival rates (Park et al. 2018). Additionally, the grade of the tumour might affect the effectiveness of the therapy. Furthermore, there are some factors that increase the chance of occurrence of CRS, such as high baseline serum ferritin (>1500 μg/L), Lymphodepletion with fludarabine-based conditioning, and severe thrombocytopenia (Hay et al. 2017, Murthy et al. 2019). Therefore, patients with these comorbidities should avoid this type of therapy.

However, localised, unlike metastatic, prostate cancer showed favourable outcomes and a high survival rate with few undesirable side effects after standard treatments. Regarding the ethical principle that doing no harm takes precedent over bringing benefits, should not do further investigations on localised prostate cancer patients by using CAR-T cell therapy until finding a way to decrease CRS events.

Conclusion

Although chimeric antigen receptor (CAR)-T cell therapy targeting prostate tumour-specific antigen has shown promising results, yet there are several issues that need to be precisely studied before applying CAR T cell therapy for prostate tumours. Further investigations to the molecular basis underlying the Immunological Complexity of the Prostate Cancer Microenvironment and the immune-related adverse effects would enhance the efficacy and diminish the side effects of this novel therapeutic approach. Also, in-depth investigations for the most specific & highly expressed prostate antigens and for the most suitable combination of antigens would significantly minimise the On-target/off-tumour toxicity. Researches should also focus on the cytokines and chemokines that could enhance the cytotoxic functions of T cells and diminish the TME immunosuppression presents in prostate and other solid tumours. Finally, the identification of predicting biomarkers for toxicities & treatment response and biomarkers that help identify the most suitable patients for this type of therapy will significantly enhance the efficacy of CAR-T cell therapy.

Disclosure statement

The authors declare that there is no conflict of interest to disclose. All authors have approved the manuscript.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Othman Mohammad Saleh (http://orcid.org/0000-0002-2194-7127)
Khaled Anwar Albakri (http://orcid.org/0000-0003-1462-4204)
Yasmeen Jamal Alabdallat (http://orcid.org/0000-0001-6855-3718)
Majd Hamdi Dajani (http://orcid.org/0000-0003-4152-0400)
Walaa Bayoumie El Gazzar (http://orcid.org/0000-0001-5172-1105)

Data availability statement

All relevant raw data will be freely available by the authors.

References


Avanzi, M.P., et al., 2018. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell reports, 23 (7), 2130–2141.


Guedan, S., et al., 2018. Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI insight*, 3 (1), e96976.


Nie, W., Ninomiya, S., Ni, J., Neelapu, S.S., 2019. CAR-T efficacy: is conditioning the key?


Ninomiya, S., et al. 2015. Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. Blood, 125 (25), 3905–3916.


Yasukawa, M., et al., 2000. Granule exocytosis, and not the fas/fas ligand system, is the main pathway of cytotoxicity mediated by alloantigen-specific CD4(+) as well as CD8(+) cytotoxic T lymphocytes in humans. Blood, 95 (7), 2352–2355.


