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REVIEW ARTICLE

CAR T-Cell Therapy: A Review of its Applications, Limitations and Future Directions

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ABSTRACT: CAR T-cell therapy is a promising avenue to explore in the treatment of cancer. Being a part of immunotherapy, it has the ability to take advantage of immunological memory to aid the immune system in attacking cancer cells with precision, which conventional treatments like chemotherapy and radiotherapy do not offer. Although it has shown successes in clinical trials, especially among children and haematological cancers, there are limitations associated with the therapy which hinders it from being widely used in cancer treatment. Phenomena like antigen escape reduce the efficacy of the treatment; adverse side effects like Cytokine Release Syndrome add on to the large cost of the treatment which renders it impractical to offer on a large scale. However, there have been proposed solutions that seek to overcome current challenges. This could be achieved by modifying the structure of the CAR, upregulating immune checkpoint pathways, or better developing management plans in the event of side effects. This article introduces the basic mechanisms of the CAR structure, explores recent successes in clinical trials and highlights future directions that will improve on the current limitations of the therapy, concluding that improved understanding of CAR T-cell therapy will enable broader implications in various cancers.

KEYWORDS: Biomedical and Health Sciences, Immunology, Immunotherapy, Cancer Immunotherapy, CAR T-Cell Therapy.

Introduction

For decades, cancer treatment has been at the forefront of scientific and medical research. According to the National Health Service (NHS), there were 288,753 new cases of cancer diagnosed in England in 2020,[1] emphasising the demand for effective care. The most conventional methods to treat cancer after surgery are chemotherapy and radiotherapy.[2] Although there have been many successes in cancer remission, there are numerous side effects that come with chemotherapy and radiotherapy, mainly because they damage healthy cells as well as cancerous ones. This potentially leads to declinations in quality of lives amongst cancer patients.[3] Although these methods continue to be used the most, there have been several recent breakthroughs leading to new therapies that can work in complement with them,[4] offering patients a wider range of options to choose from when it comes to their cancer treatment and leading to a higher success rate with combinational strategies.[5]

Immunotherapy has been explored as an alternative method by enhancing and amplifying the immune system's natural role in attacking cancerous cells effectively.[6] By using immunotherapy, tumours are able to be targeted with more precision and thus can lead to fewer side effects.[7] This therapy also takes advantage of immunological memory, which is the ability for the immune system to rapidly recognise an antigen it has previously encountered and initiate a specific immune response, decreasing the time for an immune response and minimising symptoms.[8] This results in longer remission periods, and less doses of treatment.[7]

Chimeric antigen receptor T-cell (CAR-T) therapy is one of the main types of T-cell transfer therapy, along with tumour infiltrating lymphocyte (TIL) therapy. They both require autologous transplants: T-cells are taken from a patient, then multiplied in a lab and transferred back to the same patient.[9] These two treatments differ in the laboratory process: TIL therapy involves testing which tumour infiltrating lymphocytes are best at attacking cancer cells, and growing a large number of these afterwards.[10] On the other hand, CAR T-cell therapy, a relatively new discovery, involves genetically engineering T-cells by introducing a viral vector so they produce chimeric antigen receptor (CAR) proteins on their surface. The CARs can recognise and bind to specific antigens on cancer cells, and the T-cell is responsible for destroying the cell (Figure 1).[11]

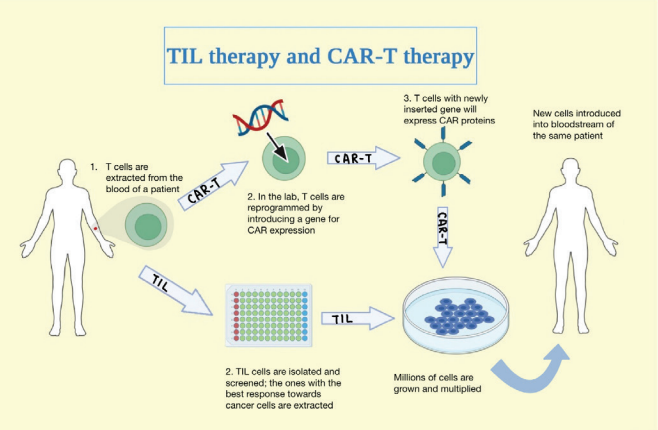

Figure 1: The pathways of TIL therapy and CAR therapy. Both treatments share various similarities like extracting T-cells from the blood of a patient, and multiplying the desired products before reintroducing them into the same patient. However, TIL cells are expanded from naturally existing T-cells whereas CAR T-cells are genetically modified T-cells, as shown in steps 2 and 3. (Created in biorender.)

DOI: 10.36838/v6i12.5

This review article aims to discuss the more recently developed CAR T-cell therapy, detailing its structure, evaluating its strengths and weaknesses, along with exploring potential avenues of improvement.

Discussion

CAR structure and function:

The CAR protein consists of 2 main parts: the ectodomain and the endodomain. The ectodomain, also known as the extracellular domain, is the region outside the membrane of the cell.[12] It has a single-chain variable fragment (scFv), a small fragment of an antibody, meaning that it can recognise and bind to different specific surface proteins or antigens attached to a cancerous cell depending on which scFv counterpart it contains.[13] The endodomain, also known as the intracellular domain, is the region inside the membrane of the cell. When the scFvs detect the corresponding antigen on a cancer cell, a signal is activated and sent through the CAR protein so that the endodomain transmits it to the T-cell, triggering mass antibody production and an immune response, which can include the release of cytokines (**Figure 2**).[14 15]

These two parts are linked by a transmembrane domain which spans the width of the cell surface membrane, serving as a connector.[15] The hinge region, an extracellular structure, is also generally included in the CAR protein which extends from the transmembrane domain. It provides flexibility and contributes to the length of the receptor in order to allow the antigen-binding domain access to the target more easily for optimal T-cell activation (**Figure 2**).[12]

Scientists have made continuous modifications to the CAR protein in order to enhance its efficacy, minimise side effects, and improve success rates, with the 5th generation CAR being the most recent one in development.[16 17] One of the most significant changes include adding or modifying costimulatory domains. They release secondary signals along with those sent by an antigen receptor, to further enhance the activation of the T-cell and improve their survival rates.[18] Other changes include modifying the scFv, hinge regions, intracellular domains, or adding accessory proteins to refine the structure and therefore function of CAR proteins (**Figure 2**).[19]

In recent generations of the CAR protein, conditional 'safety switches' or 'suicide genes' have been added, which allows for the elimination of CAR T-cells in the unexpected cases of side effects or toxicities that occur.[20] There have been many different types of 'safety switches' developed and some are even reversible, with notable success of using one named Herpes Simplex Virus Thymidine Kinase (HSV-TK) in the cases of graft-versus-host-disease (GVHD) to deactivate genetically engineered CAR T-cells in clinical trials. The HSV-TK protein turns toxic upon artificially introduced into the CAR T-cells, leading to apoptosis (**Figure 2**).[21 22]

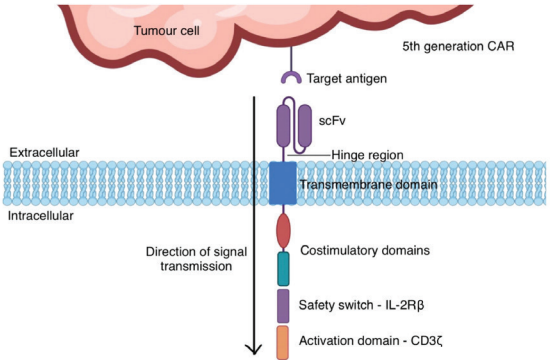

Figure 2: Structure of a 5th generation CAR across a cell surface membrane. The scFv is triggered when it recognises a target antigen on a tumour cell and sends an activation signal down the CAR T-cell to the activation domain. (Created in biorender.)

Strengths:

CAR T-cell therapy has shown many successes especially with haematological cancers, with 6 total therapies for B-cell lymphomas, B-cell acute lymphoblastic leukaemia (B-ALL), and multiple myeloma (MM) being approved by the Food and Drug Association (FDA) and the European Union as of 2024 for adults, with the first approval being in 2017. [23-25] Both associations have approved 1 drug, Tisagenlecleucel (brand name Kymriah), to be used to treat B-cell acute lymphoblastic leukaemia (B-ALL) in children and adolescents under the age of 18.[26,27] The NHS in England offers 3 CAR-T therapies for children and adults as of 2023.[28] There have been more than 10 years of data from the first batch of patients receiving the treatment in clinical trials of the 1st generation of CAR T-cells targeting Cluster of Differentiation 19 (CD19), the antigen for lymphoma, and B-cell maturation antigen (BCMA), the antigen for multiple myeloma.[24]

In a study conducted by the Lymphoma Program Investigators at the University of Pennsylvania, 24 patients with refractory B-cell lymphoma were treated with the CAR T drug tisagenlecleucel (brand name Kymriah). 46% of the 24 patients had a complete response (CR), which is the "disappearance of all target lesions"[29] or clinical evidence of the disease after the treatment was complete, over a median of 60 months following treatment. Progression free survival, which measures the time from "randomisation or initiation of treatment to the occurrence of disease progression or death",[30] was observed in 31% of patients. 60% of patients also had a sustained response, referring to a clinical endpoint conveying an absence of lesions over a set period of time, at the end of the 5-year study.[31] Another study conducted by the university reported a complete and sustained remission in 2 patients that received CD4+ CAR T-cells as part of a phase I clinical trial to treat their lymphocytic leukaemia in 2010, and these cells were still detected in their blood a decade later (**Table 1**).[32]

A study published in the Nature Journal of Clinical Oncology in April 2023 observed a 58% CR rate amongst 43 patients that received axicabtagene ciloleucel (brand name Yescarta) for B-cell lymphoma. 76% of those in CR went on to remain in long-term remission, with a decrease or complete disappearance in cancerous signs and symptoms after more than 10 years (**Table 1**).[33]

From 12 studies reporting on the outcome of CAR T-cell therapy for patients with B-ALL, there have been a range of CR rates from 62% to 86%. Patients that were administered commercial drugs like tisagenlecleucel and brexucabtagene (brand name Tecartus) had a median of 5.6 months of progression free survival. However, it is important to note that there was a large range of progression free survival rates (13-88%) between the studies, and it should be taken into account that some patients received a haematopoietic stem cell transplant after treatment which may increase the progression free survival rate (**Table 1**).[24]

Studies also show the increase in successes of CAR T-cell therapy amongst younger patients, especially in relapsed or refractory acute lymphoblastic leukaemia. The ELIANA global trial reported an overall remission rate (ORR) of 81% in 79 paediatric and young adult B-ALL patients after tisagenlecleucel was administered; 95% of these were minimal residual disease (MRD) negative by day 28 after initial infusion,[34] meaning that no evidence of the malignancy was detected post-treatment (**Table 1**).[35]

There is less long term follow up data on patients with MM who received CAR T-cell therapies that target BCMA; this is largely due to the recent development of this specific CAR.[24] In a four-year follow up study in China of the CAR-T drug axicabtagene ciloleucel administered to patients with relapsed or refractory (r/r) MM, there was a 73% CR rate amongst the 74 patients. ORR was at 88% and the progression free survival was reported to be at 18 months.[36] However, despite the successes these trials may show, there have been a large proportion of patients from these studies developing side effects or associated toxicities after treatment. (**Table 1**)

Table 1: Table of various CAR T-cell therapy clinical trials and their respective outcomes. (Created in Canva.)

Trial	Malignancy	Drug	Result
Lymphoma Program Investigators at the University of Pennsylvania	B-cell lymphomas	Tisagenlecleucel (Kymirah)	46% complete response 60% sustained survival 31% progression free survival
Nature Journal of Clinical Oncology	B-cell lymphomas	Axicabtagene Ciloleucel (Yescarta)	58% complete response 76% of those in long term remission
Collective from 12 studies; outlined in nature reviews clinical oncology	B-ALL	Tisagenlecleucel (Kymirah) and Brexucabtagene (Tecartus)	62-86% complete response 5.6 months progression free survival at 13-88%
ELIANA global trial for paediatric patients	B-ALL	Tisagenlecleucel (Kymirah)	60% complete response 81% overall response rate 95% minimal residual disease negative by day 28
LEGEND-2 trial in China	r/r MM	Axicabtagene ciloleucel (Yescarta)	73% complete response 88% overall response rate 18 months progression free survival

Limitations and potential strategies:

The development of CAR T-cells has been a remarkable breakthrough in modern science and has shown positive results in clinical trials. However, there are many serious scientific limitations that reduce its efficacy in bringing about remission, impeding it from being used on a wider scale in cancer treatment.[37] In a systematic review and meta-analysis report on 12 month follow up studies collected by Zinzi *et al*, 61% of patients on average experienced relapse within a year of receiving CAR T-cells.[38] Moreover, according to Gu *et al*, "40% to 60% of CR patients eventually experience relapse" after using CAR-T to treat B-cell malignancies.[39]

Antigen escape is one of the main factors that accounts for relapses in malignancies shown in clinical trials and is a major limitation in the further development of CAR T-cells.[39,40] Although CAR T-cells are very effective at targeting specific antigens expressed on malignant T-cell surfaces that they are programmed to recognise, this phenomenon occurs when some of these cancerous cells present a change in antigen displayed on its surface, evading attack. [41] As a potential approach to prevent relapse, the use of multi-antigen-targeted CAR T-cells is currently being experimented. Several of these methods proposed by scientists include co-administration in which two CARs are infused for two different specific antigens, and dual specificity in which one CAR contains two or three scFvs. This allows the recognition of more than one antigen: T-cells are activated when either of the antigens are encountered, and when all antigens are present simultaneously the immune response is further strengthened. Even when one antigen has escaped CAR recognition, there would still be a higher chance in triggering an immune response due to the identification of other antigens on the cancerous cell's surface.[40, 42-44]

Although CAR T-cell therapy has shown success in treating haematological malignancies like leukaemia, lymphoma and multiple myeloma, the extension of this therapy to tackling solid tumours is still in development. Key reasons for this include on-target-off-tumour effects, limited T-cell trafficking and infiltration, and the immunosuppressive tumour microenvironment.[45]

Antigens on solid tumours are often also present on healthy tissues to different degrees which is why the involvement of CAR-T in targeting solid tumours presents such a challenge.[41] This can be described as an 'on-target-off-tumour' effect, in which 'on target' refers to the specific antigen found on the surface of cancer cells that CAR T-cells are designed to target, and 'off tumour' means that the CAR T-cells also recognise the same antigens on normal cells.[46] For example, the tumour associated antigen (TAA) human epidermal growth factor receptor 2 (HER2), used widely as a target antigen in CAR T-cell therapy, is present at high levels on cancer cells and is responsible for promoting rapid growth and metastasis of tumours.[47] However, this TAA is also present on epithelial tissues at low levels. This 'on-target-off-tumour' effect results in normal tissues also being harmed or killed, which leads to detrimental effects on the patient's health, like neurotoxicities.[48]

A solution already being put into place for this is the engineering of 'off switches' or 'suicide genes' into CAR T-cells that allow CAR signaling to be regulated after infusion. [49] One common 'suicide gene' system consists of an inducible Caspase 9 (iCASP9) gene engineered to be incorporated into the CAR. A small molecule drug, such as AP1903 is administered to a patient intravenously in cases of toxicities. AP1903, a chemical inducer of dimerisation, binds specifically to the iCASP9 domain, triggering the formation of the dimer caspase 9. This

DOI: 10.36838/v6i12.5

induces apoptosis of the CAR T-cell so it does not continue inducing toxicities of any kind.[50] Another potential strategy to avoid 'on-target-off-tumour' effects is called affinity tuning. A scFv with high affinity on a CAR means that it is more sensitive to antigens and thus can strongly bind to cells with antigens at any level, potentially attacking non-malignant cells as well as malignant ones. On the other hand, a scFv with a low affinity binds weaker to any antigen presenting cell, making it easy for tumour cells to escape. It is key to find a balance in the affinity of the scFv so it can distinguish differences between tumours with a high density of target antigens, and normal healthy cells with a low density of target antigens.[20, 51]

Another presenting barrier towards the efficacy of attacking solid tumours is the limited trafficking and infiltration of the CAR T-cell into tumour tissue. There are physical barriers in solid tumour masses like the dense fibrotic matrix, tumour stroma and surrounding vasculature, which proves difficult for CAR T-cells to penetrate through and successfully reach the target location.[52] In addition, solid tumour tissues express many different chemokines at low levels which send mixed signals towards CAR T-cells, therefore impeding accurate migration towards cancer cells.[45] Chemokines are a group of small proteins that signal the movement of cells, especially white blood cells, in the immune system during immune responses. Tcells contain chemokine receptors which receive these signals from a malignant cell and thus migrate towards it.[53] A potential solution to increase trafficking is to administer CAR T-cells locally instead of systematic delivery. This not only limits the need for CAR T-cells to travel a large distance to the target tumour region, decreasing the probability of encountering traffic, but also reduces 'on-target-off-tumour' toxicities as there is less interchange with healthy tissue.[20, 41] Moreover, as most of the movements of CAR T-cells are heavily connected to chemokines, the understanding of them in solid tumours can be very advantageous to designing of future generations of CARs. By distinguishing specific chemokines released by their respective solid tumours and defining their expression and behaviours, scientists can engineer chemokine receptors on CARs with more specificity (**Figure 3**).[54]

Lastly, the immunosuppressive tumour microenvironment (TME) also provides a challenging environment for all immune cells, including CAR T-cells to survive in and navigate to the target site. Surrounding the solid tumour, immune cells, blood vessels and a complex extracellular matrix are amongst the myriad of non-malignant cells that make up the TME.[55] Even though immune cells usually carry out successful immune responses, in this case they are reprogrammed by the tumour to promote tumour growth instead of tumour suppression.[56] This is achieved by the secretion of immunosuppressive cytokines to specifically suppress the immune cells' respective functions. This creates a muted immune response and the immune cells contribute to the tumour mass instead.[57] Immune checkpoint pathways such as PD-1 or CTLA-4, crucial for moderating immune responses, are upregulated and thus cause an increase in immune cell death.[41] Moreover, the interaction of components in the TME hinder the effectiveness of the immune system and aids the tumour to escape through various functions.[58] For example, the TME alters its metabolic environment so T-cells have to undergo anaerobic glycolysis instead of oxidative phosphorylation to release ATP for respiration. This process, called the 'Warburg effect', causes T-cell exhaustion as anaerobic glycolysis generates 18 times less ATP; T-cells are thus not able to infiltrate the microenvironment completely and locate the tumour target due to their lack of energy.[59] T-cell exhaustion is common in CAR T-cell therapy, it is characterised by a 'decreased expression of effector cytokines and increased expression of inhibitory immune checkpoint receptors'.[60] Moreover, the TME is a region of low oxygen supply due to its irregular blood flow. Therefore, anaerobic respiration of the cells in the TME produces lactic acid that increases the acidity in the microenvironment due to hypoxia. Whilst malignant cells are able to survive in these conditions, it is the opposite for T-cells and they can only persist in the TME for a limited time (**Figure 3**). [59, 61]

To reduce T-cell exhaustion and target solid tumours with more success, strategies are needed for CAR T-cells to survive in tumours. At present, the use of immune checkpoint blockade is the most promising method in reversing the effects of the upregulation of immune checkpoint pathways.[62] Immune checkpoint blockade utilizes drugs that target PD-1 or CTLA-4 by blocking checkpoint molecules from binding with receptors on CAR T-cells.[63] A drug for checkpoint blockade therapy, ipilimumab (brand name Yervoy), was first approved by the FDA in 2011 for melanoma. Since then, there have been many other antibody drugs experimented in clinical trials and have shown great success.[64] In addition, combination therapy is also widely used in relation to CAR-T therapy to better target solid tumours. Chemotherapy is used as a 'bridging therapy', meaning that after T-cells are taken from the host patient, chemotherapy is administered during the waiting time period of about 45 days before infusion. This inhibits further tumour growth and stabilises the patient's condition. It can also weaken the solid tumour and its surrounding TME, potentially decreasing T-cell exhaustion and enhancing the efficacy at which CAR T-cells can target the malignancy.[65, 66]

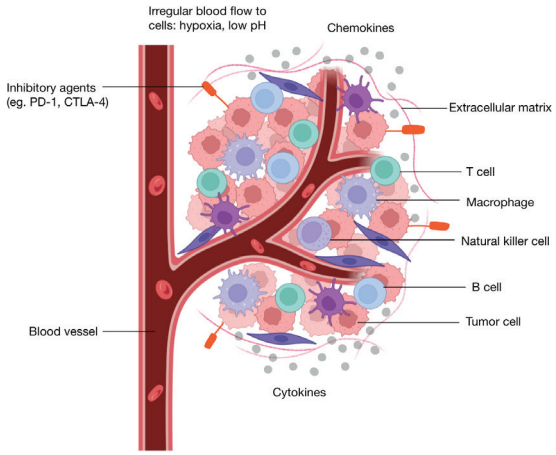

Figure 3: The tumour microenvironment is hostile to CAR T-cells given its hypoxic and acidic environment which causes T-cell exhaustion. The presence of a dense and abnormal extracellular matrix, inhibitory agents, chemokines and tumour-associated immune cells also limit T-cell trafficking and infiltration. In addition, the upregulation of cytokines causes many side effects. (Created in biorender.)

Side effects:

In addition to CAR T-cell weaknesses, the serious side effects that CAR-T therapy induces is also one of the many reasons why this procedure is not often carried out in cancer treatment.[67] Side effects include neurotoxicities, infusion associated infections, and most commonly, cytokine release syndrome. Therefore, patients have to be kept under careful monitoring, and timely interventions have to be made to manage various side effects after infusion.[68]

Cytokine release syndrome (CRS) is an adverse side effect that occurred in up to 93% of patients across several clinical trials, affecting 9 in 10 patients that receive CAR T-cell therapy. [69] CRS is caused by the elevated release of cytokines as a response to inflammation after infusion.[70] This occurs within the first two weeks after infusion, usually lasting for a further two to three weeks depending on the severity and treatment.[71] CRS can manifest in a range of symptoms, with the first clinical sign being a fever that can escalate up to 40.5°C (104.9°F).[71] Other symptoms are often flu-like, which include headaches, rashes, nausea and a cough. However, in severe cases, there can be a presence of acute respiratory distress syndrome (ARDS), multi system organ failure, hypoxia, circulatory shock and unexpected death.[72]

Another common toxicity associated with CAR T-cell therapy is neurotoxicity, in particular immune effector cell-associated neurotoxicity syndrome (ICANS). This manifests in up to 67% of patients and often arises with CRS. Symptoms are mostly neurological like aphasia and apraxia; severe toxicity can result in cerebral edema, seizures and fatal intracerebral haemorrhage. The mechanisms of ICANS are not fully understood, so further research regarding its pathogenesis is required in order to better develop treatments.[69, 73]

Both toxicities have individual grading scales from 1-4, with 1 being the mildest and 4 being the most severe. Treatment and management plans are mostly based on the severity and thus require cautious monitoring of symptoms. Symptomatic treatment is mainly adopted, like the administration of anti seizure medication. Magnetic resonance imaging (MRI) scans of the brain are obtained in ICANS to further examine the patient and identify biomarkers.[68, 74]

Tocilizumab (brand name Actemra), a drug that inhibits the cytokine interleukin-6 (IL-6), has been used for treating both CRS and ICANS by decreasing inflammatory reactions in low grade toxicity syndromes. Although partially successful, the most efficient timings and dosages of administering Tocilizumab have not been fully ascertained yet.[75, 76] Moreover, corticosteroids, synthetic hormones that decrease inflammation, have commonly been used in combination with Tocilizumab in higher grades and have further decreased severe side effects.[77, 78]

Nevertheless, these two drugs are still insufficient in tackling the extensive cytokine storm. Other strategies that are currently being investigated include 'suicide genes'[75] and kinase inhibitors that reduce signalling pathways involved in cytokine production.[79]

Another complication associated with CAR T-cell therapy is the risk of infection, which can happen before or after infusion. Common infections include bacterial and viral infections.[80, 81] This is not only due to the risks associated with treatment-related invasive procedures, but also because of the patients' weakened immune systems both prior to and post infusion.[82] Before treatment, many patients undergo chemotherapy as a 'bridging therapy' in order to suppress further tumour growth and weaken the hostile TME.[83] Although this reduces CAR T-cell exhaustion and may result in a more successful treatment, chemotherapy also causes immunosuppression and a disruption in the composition of their microbiome. This can lead to an increased risk of developing infections even before CAR T-cells are infused.[84] One common strategy to reduce infection is for patients to undergo immunoglobulin replacement therapy both before and after CAR-T infusion, which provides more antibodies to fight infection.[85] Vaccinations could also be administered at least two weeks prior to infusion for prevalent diseases at the time: for example, the influenza vaccine during the influenza season. The type of vaccine given differs from patient to patient and each case should be individually considered.[86]

At the end of 2023, there had been 22 reports of rare secondary T-cell malignancies reported to the FDA. They were reported in patients who had received CAR T-cell therapy in the form of B-cell maturation antigen (BCMA) and CD19, with 14 of these cases arising within 2 years after infusion.[87, 88] Whilst a direct causation has not yet been identified, the FDA has seeked to issue warning labels detailing this on approved CAR-T drugs.[89] The FDA is still investigating this correlation as of May 2024 and is 'evaluating the need for regulatory action'.[90]

Problems regarding cost:

A significant barrier to CAR T-cell therapy is the high price: the price of a CAR-T drug for a patient ranges from $373,000 to $475,000 which raises concerns about affordability and accessibility amongst different healthcare systems.[91] Moreover, an additional cost of more than $500,000 is required for hospital monitoring and adverse event management after infusion, resulting in the whole procedure exceeding $1 million.[92] Since there is a lack of clinical data to observe success rate, it is hard to evaluate the costeffectiveness of the therapy. This is especially true for data regarding long-term remission and outcomes given that the therapy has only been in use for about a decade.[93] The reasons for this costly treatment lies mainly in the manufacturing processes, transport procedure and the patient tailored nature of CAR Tcells.[94] Firstly, the manufacturing process is long, involving highly skilled personnel and a controlled environment. Complex procedures like leukapheresis - separating white blood cells from the blood to obtain T-cells - require advanced technology and thus increases the price of the drug.[95] Transportation costs also are a challenge to both the manufacturers and patients: manufacturers have to use a method called cryopreservation, a process that freezes CAR T-cells to prevent any damage occurring to their cell structure, when sending the final product back to the infusion site.[96] Patients also may have to travel far to receive their infusions as CAR-T therapy is currently only available in a

DOI: 10.36838/v6i12.5

limited number of large medical centres or hospitals.[67] Most pressingly, the therapy is based on autologous T-cells, meaning that the patient receives their own modified CAR T-cells. This patient-tailored approach greatly increases the cost of CAR T-cell therapy as it is laborious and time-consuming, as the genetic engineering process has to be done for each individual batch of T-cells.[24]

A proposed strategy to make CAR T-cell therapy more accessible is the opening of more treatment centres and to increase the scale of CAR-T production in order to lower transport costs and reduce the time between manufacture and infusion.[67] Another widely proposed strategy involves the use of allogeneic CAR T-cells, transplanting cells from one person to another, to lower the price and enable this treatment to be more affordable. Allogeneic CAR T-cells are provided in large amounts from healthy donors which enables manufacturers to supply modified T-cells to patients relatively quickly and efficiently for infusion instead of having a long waiting period. This not only reduces the cost of production but also prevents further deterioration of the patients' health by saving time. However, allogeneic transplants pose the risks of GVHD and allorejection due to the transplantation of 'foreign cells,' which therefore hinders the transition from the use of autologous to allogeneic cells. There are ongoing research efforts to reduce the likelihood of this phenomenon like gene editing technology in the form of CRISPR/cas9.[97]

Ongoing research efforts:

As there are numerous challenges interfering with the efficacy of CAR T-cell therapy, there is a lot of ongoing research being conducted to tackle these barriers.

The problem of tackling solid tumours has been a predominant one in the limitations of CAR T-cell therapy. In order to expand its use to solid tumours, there are several obstacles that need to be addressed first.[45] There have been continuous modifications to the CAR structure to improve its ability to recognise its targets:[17] the co-expression of several scFvs on a CAR protein has been proposed as an avenue to prevent antigen escape and tumour antigen heterogeneity in solid tumours.[40] This multi-antigen targeted approach will allow the CAR T-cell to recognise different phenotypes of tumours and enhance its efficacy and specificity.[42] In addition, there has been much progress in refining the 'suicide genes' or 'offswitches' on CAR proteins in order to minimize the effects of the 'on-target-off-tumour' phenomena.[20]

With solid tumours, T-cell trafficking and exhaustion are very common reasons for its limited efficacy in migrating towards malignant tumours.[52] Scientists have looked at administering CAR T-cells locally instead of systematically in order to reduce trafficking and decrease the distance T-cells have to travel before reaching the tumour site.[41] The use of immune checkpoint blockade and combination therapy have produced promising results in tackling the hostile TME,[62] but further strategies are needed to amplify the ability of CAR T-cells in infiltrating the surrounding microenvironment of the solid tumour.

Side effects and patient safety are important problems to address in CAR T-cell therapy as well.[67] Although drugs like Tocilizumab and corticosteroids have decreased inflammatory reactions, their dosages have not been fully ascertained and there has been limited success in managing side effects.[75,76] Therefore, more research into the development of associated side effects like CRS and ICANS has to be conducted in order to develop better treatments and potentially even prevent adverse side effects from manifesting in the first place.[69,73] Scientists have also looked into modifying the structure of CAR proteins and have included 'off-switches' to tackle the cytokine storm. Other modifications to the proteins are also continuously under consideration.

The FDA is currently looking into reports of secondary T-cell malignancies following CAR T-cell therapy, although they only represent a small fraction of reported side effects.[90] As there is a lack of long term data since the therapy is relatively new, further data collection is required to gain an insight into long term effects.[24]

Alternative manufacturing methods like the use of allogeneic CAR T-cells are also currently under research in order to lower the cost of this therapy and make it more accessible and affordable. However, this strategy comes with challenges of its own and thus its use has to be carefully considered.[97]

■ Conclusion

In conclusion, CAR T-cell therapy is a promising avenue in using immunotherapy to treat cancer and has shown successes in clinical trials and in early treatment, especially with haematological malignancies. CAR T-cell therapy not only allows tumours to be targeted more precisely, but it also leads to longer remission periods due to the activation of immunological memory. Most significantly, CAR T-cell therapy has shown prominent success in relapsed or refractory cancers. However, there are still weaknesses that limit its applications in solid tumours. In addition, CAR T-cell therapy comes with adverse occurrences and side effects which deter many from integrating it into their personal treatment. Since this therapy is a highly personalised approach and requires complicated manufacturing methods, the problem of cost is also a major barrier. There is a significant amount of research and funding dedicated to tackling these challenges; different strategies and modifications to the structure of the CAR protein and the process of the therapy have been suggested. With the continuous improvement of CAR T-cell therapy and further research into the mechanisms surrounding the therapy itself, and with the accumulation of more data surrounding effects after treatment, it is hopeful that CAR T-cell therapy will be more integrated into cancer treatment along conventional therapies and will be more accessible to all.

■ Acknowledgments

The author is grateful to her mentor, Collin McColl for his continued guidance and support throughout the research and writing process. Figures in the review article were created by the author with the platforms Biorender and Canva.

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DOI: 10.36838/v6i12.5