CAR-T CELL THERAPY FOR CANCER TREATMENT

By

Masuma Aktar
20346027

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy
Brac University
June 2024

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

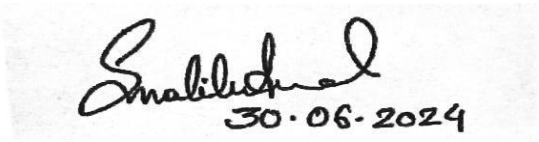
Student's Full Name & Signature:

Masuma Aktar
20346027

Approval

The thesis titled “CAR-T cell Therapy for Cancer Treatment” submitted by Masuma Aktar (20346027) of summer, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on June 2024.

Supervised By:



Syeda Maliha Ahmed

Lecturer

School of Pharmacy

Brac University

Approved By:

Dean:

A.F.M. Yusuf Haider, PhD

Acting Dean, School of Pharmacy

Professor, Department of Mathematics and

Natural Sciences

Brac University

Ethics Statement

The project does not involve any use of animal models. Hence, no animals were harmed in the process. Additionally, there was no involvement of human participants as well, and thus, informed consent is not applicable.

Abstract

Chimeric Antigen Receptor (CAR)-T cells are one of the most promising innovations in cancer treatment. CAR-T cells are particularly suitable for treating hematologic cancers, including leukemias and lymphomas. Experimental T-cells are modified genetically and show antennas definite to cancer cells. It may, therefore, detect cancer cells more easily and destroy them. In a patient, the T-cells are extracted, carriers of CAR are injected in vitro, and the altered cells are reinfused through the blood. The procedure has expressed outstanding outcomes in certain leukemias and lymphomas, with recuperative rates in patients who have completed all available conventional therapies. Despite this, some difficulties remain, like cytokine explosion syndrome, neurotoxicity, and low efficacy in solid tumors. This review aims to provide a brief overview of the current status of CAR-T cell therapy and discuss prospective future implications of the treatment in cancer.

Keywords: Chimeric Antigen Receptor (CAR)-T cells; leukemia; lymphomas; tumors; cancer cells.

Dedication

The project work was dedicated to my parents and teachers.

Acknowledgement

I would like to thank all the members who helped in finishing this research. I am thankful to Allah for being part of this project with such a polite and helpful team from the School of Pharmacy, BRAC University. I would like to convey my sincere appreciation to Professor Dr. Eva Rahman Kabir & the Dean of the School of Pharmacy and Professor Dr. Hasina Yasmin, the Assistant Dean, program Director of the School of Pharmacy for providing me such beneficial knowledge and constant encouragement & inspiration. I wish to acknowledge my appreciation to my supervisor Syeda Maliha Ahmed, Lecturer at the School of Pharmacy, for every support she rendered towards my work. I felt happy and privileged to be able to work under her supervision. I would also like to thank all the faculty members of School of Pharmacy, BRAC University and my friends and seniors who helped me out during this thesis writing and my family members for their unbounded support.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ALL	Acute Lymphoblastic Leukemia
BCCR	Breast Cancer Collaborative Registry
BCL	B-cell lymphoma
CAR	Chimeric Antigen Receptor
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CIS	Carcinoma In Situ
CEA	Carcinoembryonic antigen
DLBCL	Diffuse Large B Cell Lymphoma
EGFR	Estimated glomerular filtration rate
FDA	Food and Drug Administration
HSCT	Haematopoietic stem cell transplantation
MHC	Major Histocompatibility Complex
NCI	National Cancer Institute
NHL	Non-Hodgkin Lymphoma
PSMA	Prostate-specific membrane antigen
PMBCL	Primary mediastinal large B-cell lymphoma
TFL	Transformed follicular lymphoma

Chapter 1

Introduction

1.1 What are CAR-T cells?

Chimeric Antigen Receptor (CAR)-T cell therapy represents a type of immunotherapy that involves modifying the cells of the patients in a lab so that they can identify and destroy the cancer cells that have particular proteins called antigens. T cells possess a notable capacity to fight infections. Using CAR-T cells in cancer treatment, especially hematologic malignancies, is an important advance in cancer treatment. This innovative approach targets cancer cells by modifying a patient's T cells genetically so that they can produce a CAR therapy. Despite its early success in blood malignancies, the area of CAR-T cell therapy has expanded rapidly since its first FDA approval in 2017. From a projected $2.3 billion in 2022 to a predicted $10.3 billion in 2030, the worldwide market for CAR-T cell therapies is desired to experience a growth rate of over 21% annually (Singh et al., 2023).

Patients with B-cell malignancies, such as non-Hodgkin lymphoma (NHL) and relapsed or resistant acute lymphoblastic leukemia (ALL), have shown exceptional response to CAR-T cell therapy. By focusing on the clinical trials, as many as 90% of patients had full remission. There have also been long-lasting effects, which could lead to permanent illness control or even a cure in extreme circumstances (Porter et al., 2021).

The CAR-T cell treatment approach involves multiple steps as illustrated in Figure 1. At first, the blood of a patient is drained of its T cells. The second step is to modify these T cells in a lab by inserting the gene for a specific receptor known as a CAR. The patient's T cells are transformed into CAR-T cells when the gene that encodes the modified CAR protein is showed on their surface. In vitro cultivation of millions of CAR-T cells is a general practice. The patient

is administered these via intravenous infusion. Then CAR-T cells attack the cancer cells by binding to their antigens (Mount et al., 2018).

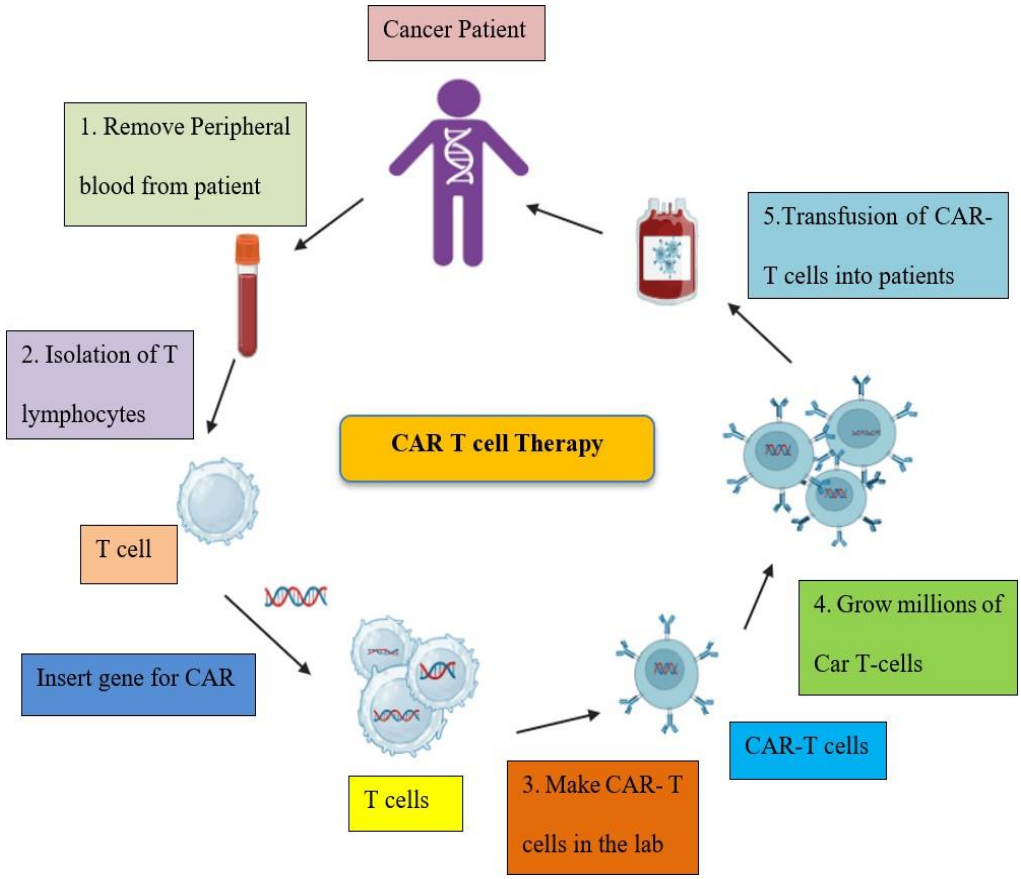


Figure 1: Treatment of Cancer using CAR-T Cell Therapy

The path of CAR-T cells starts with sampling T lymphocytes from the blood of patient. After that, they are shipped to the appropriate laboratory, where they are converted. A viral vector that carries a genetic plan for a CAR is injected into the T cells. The receptor itself was created to see a targeted antigen in the best way feasible and is often present on the outside of cancerous tissue. It is integrated into the DNA of the T cells, and they are endowed with a new receptor. The CAR-T cells are ready for infusion back into the patient after being reproduced to the millions (Mohamed Reda Benmebarek et al., 2019). After being reintroduced into the blood, CAR-T cells undergo a search-and-destroy process. They have been modified to help their artificial receptors attach to antigens in cancer with exceptional specificity. As a result, the

connection between cancer antigens and CAR-T cells' receptors is the most important for initiating killing. CAR-T does not need a Major Histocompatibility Complex (MHC) for the introduction of antigens, unlike natural T cells, which provide CAR for destroying cancer cells that are resistant to the humoral type of immune response. Once the CAR-T binds with the targeted cancer antigen, there is a chain of signaling incidents in the cells. These processes activate the cells and initiate the release of cytotoxic substances into the cancer cell, such as granzymes and perforin. The components make pores in the cell membrane of cancer and promote apoptosis, killing the cancerous cell. Secondly, the activated cells start to multiply, increasing in number to improve the elimination of cancerous cells (Watanabe et al., 2021).

1.2 History of CAR T cells

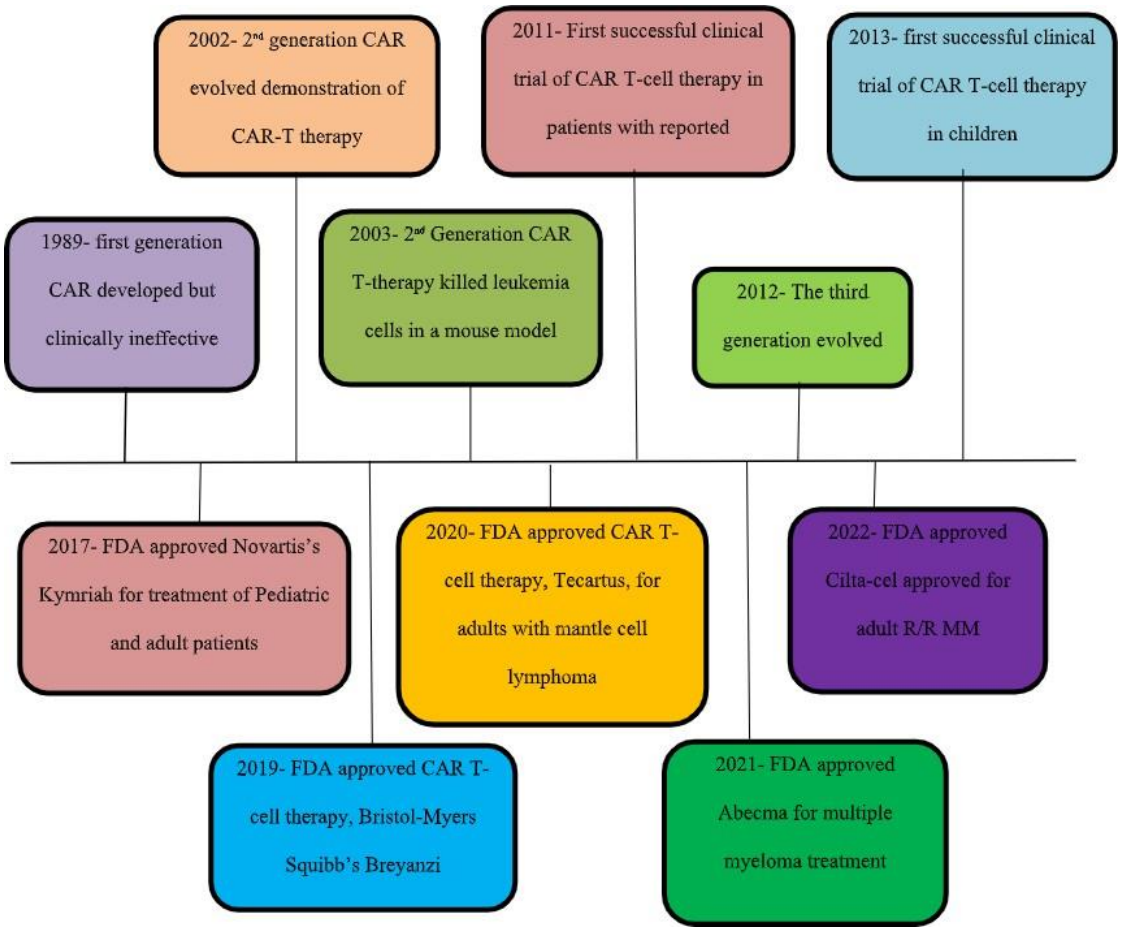


Figure 2: History of CAR-T Cell Therapy

Although the topic of CAR-T cell therapy has been talked about for several decades, the method itself appeared quite recently. In the 1980s, the genetically engineered T-cell receptor was discovered. It became known that the T-cell can recognize the cancerous cell itself and fight only against it. Unfortunately, at that time, such a method was far from ideal. It was required that the exact antigen of the cancerous cell be found, and based on this antigen, the T-cell receptor was created. Zelig Eshhar and team created the first CAR today in the first version using a monoclonal antibody with signalling. Pehlivan, Duncan, and Lee state that such CAR-T cells, having originated as early as 1989, do not need MHC for detecting cancerous cells. That is why this T-cell is more universal in comparison with T-cell engineered (Schepisi et al., 2020). The history and evolution of the CAR-T cell therapy are shown in Figure 2.

The first reason why the first-generation CARs could not significantly target cancer cells is first-generation CARs did not have the co-stimulatory actions that were critical for T-cell proliferation and survival. When this was established, the idea of changing T-cell specificity through chimeric antigen receptors was conceived. Towards the mid-1990s, researchers designed CAR of the second generation. The urge to conduct clinical trials for CAR-T cell treatment was supported by the enthusiasm of cancer researchers induced by impressive results obtained in preclinical animal models of daring designs of CAR. In 2002, the first CAR-T cell with a CAR made from the CD28 co-stimulatory domain was made (Shen et al., 2019). In 2003, investigators demonstrated that CAR-T cells could irradiate B-cell lymphoma in mice by targeting a B-cell antigen, CD19. Scientists studying the CAR-T cell treatment can base the present study on these findings. A clinical trial was conducted for the first time in 2006, carried out by the National Cancer Institute (NCI) under the supervision of Steven Rosenberg and his research team, which was successful (Mount et al., 2018).

In this study, a participant with metastatic renal cell carcinoma was administered the trial's autologous T-cells engineered to indicate a chimeric antigen receptor CAR that targeted the

carcinoma antigen carbonic anhydrase IX. Although it was a safe treatment, it did not markedly improve the patient's clinical condition. Clinical trials have focused on two primary hematologic malignancies: leukemia and lymphoma. As a result, June and other researchers at the University of Pennsylvania successfully treated patients with chronic leukemia CAR-T cells in 2011. The aborted study that utilized autologous T-cells that expressed CARs killed three people, who all recovered a long-term, complete remission. As a result, the outcomes of CAR-T cell therapy in clinical trials have contributed to the current use of adoptive cell therapy in leukemia and myeloma treatment. The primary trial that demonstrated successful CAR-T cell therapy on children with recurrent or chemotherapy-resistant acute lymphoblastic leukemia BCAL was conducted in 2013 at BCCR. This trial was performed on two children, and their T-cell cells were genetically engineered (Tchou et al., 2017). Both of them recovered a long-term, complete remission after receiving CARs that focused on the anti-CD19-BCAL antigen. In 2014, Adusumilli and colleagues developed CARs' ability to detect mesothelin in solid tumors. In 2015, researchers at BCCR improved the fourth generation of CARs. In 2017, Sadalain's group began making CRISPR-edited CAR-T cells. In that same year, the FDA approved the assistance of Tisagenlecleucel Kymriah as adoptive therapy for cancer patients who were already undergoing radiotherapy; this was another critical advancement (Schietinger & Greenberg, 2024).

The objective of this review is to give a brief overview of CAR-T cell therapy, their current status in cancer treatment and future implications.

Chapter 2

Architecture of CAR-T cells

The concept of CAR T-cell therapy was proposed in the late 1980s. These cells possess an internal structure that is divided into ectodomain, which contains antigen-binding fragments, a spacer region, an internal segment and a transmembrane domain. CAR T cells' basic modular organization has changed little since their inception, and there have been five generations of development founded on the intracellular triggering field configuration (Schepisi et al., 2020). The structural variations from the 1st to the 4th generation of the CAR-T cell design are shown in Figure 3.

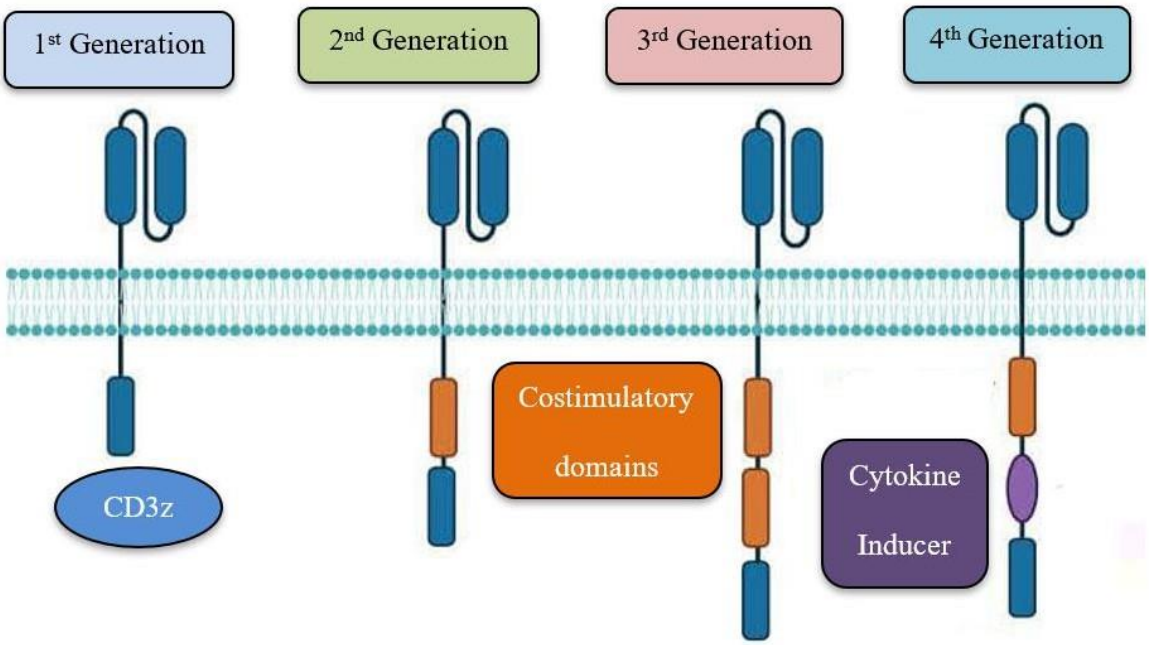


Figure 3: Structural Variants of Various Generations of CAR-T Cells

The 1st generation: The initial CARs were equipped with either a solitary CD3 ζ-chain or a FcεRIγ intracellular domain and did not possess any supplementary costimulatory domains. Practically, the complexes were likely to have the natural TCR and had the same drawbacks, such as the incapability to produce the necessary quantity of IL-2. It is widely recognized that a weak response can be addressed by adding exogenous IL-2 to first-generation CARs to get

the desired impact. In addition, despite being modified, the cells exhibited limited cell growth and short in vivo survival. As a result, the formation of costimulatory regions was initiated (Neelapu et al., 2017).

The 2nd generation: Incorporating the dual signalling that leads to high T-cell proliferation in the natural process, the second generation was designed to solve these shortcomings. They have extra cytoplasmic domains like OX-40, CD28, or 4-1BB, either alone or in combination, to reverberate a secondary signal when they encounter a cancer antigen. Results from both animal studies and human trials indicated that the existence of costimulatory signals increased cytotoxicity and proliferation while also providing a longer half-life in living organisms, leading to a more sustained response. These characteristics were also impacted by the costimulatory domain features (Ramos & Gianpietro Dotti, 2011). Some research has suggested that 4-1BBζ cells might have a higher half-life in the bloodstream compared to CD28ζ cells. The distinguish between the two is that the former results in constitutive activation and the latter causes CAR-T cells to exhaust early. Improved costimulatory constructions have resulted from this insight (Roybal et al., 2016).

The 3rd generation: A 3rd generation CAR with many costimulatory signalling domains in its endodomain was responsible for this accomplishment. Examples of these structures are marked as CD3ζ-CD28-OX40 or CD3Ŏ-CD28-41BB. Since 4-1BB endodomains promote long-lasting CAR expression and CD28 costimulatory domains are known to permit rapid tumor termination, the latter was determined to be particularly promising. While their application in cancer treatment has been fruitful leading to fewer side effects and better persistence and proliferation has surpassed the results achieved with second-generation (Ritchie et al., 2013).

The fourth generation: Based on second-generation constructions, fourth-generation CARs were designed since adding additional costimulatory domains did not improve the activity of CAR-T cells. A transgenic protein, like a cytokine, is added to the second generation using an inducible or constitutive expression cassette, which is the sole difference. The goal of these chimeric antigen receptor T cells is to kill universal cytokine-mediated, and facilitate the transportation of transgenic products to the specific location of the tumour (Roex et al., 2020).

The common method for accomplishing this is to insert the nuclear factor of activated T cell (NFAT) responsive cassette into these cells. The targeted target is contacted by CD3ζ-containing CARs, which stimulate transgene expression. Transgenic cassettes encoding the CAR structure and the cytokine are transferred during the creation of TRUCK CAR-T cells, rendering them conditionally expendable. In preclinical models, CAR-T cell therapies were far more effective than second-generation CARs due to the cytokine transgene. Another advantage of this method is that it prevents toxicity, which is the general side effect of the treatment (Rurik et al., 2022).

Chapter 3

Advantages and Limitations of CAR-T therapy

3.1 Advantages of CAR-T therapy

CAR-T cell therapy is a progressive and innovative method in the area of cancer treatment that provides hope for patients with some types of cancer. This personalized immunotherapy has numerous advantages over traditional methods and existing therapies.

Targeted Precision: Primarily, it is characterized by a high specificity of action on cancer cells. Common treatments, including chemotherapy and radiation, are destructive and can affect not only harmful cells but also healthy ones. In contrast, CAR T-cells search for the so-called antigens on the shell of cancerous cells to which they will bind and do not allow the tumor to reproduce. Thus, the normal functioning of tissues is not impaired (Mount et al., 2018).

Durable responses: Persons with refractory or relapsed malignancies have achieved long-term remissions, which is positive news on the potential of this therapy. For some patients, the period of remission could last for years, granting them the quality of life obtained through other kinds of therapy (Schepisi et al., 2020).

Rapid Recovery: Additionally, the therapy process is short since the patient receives only one infusion of the engineered cells. Therefore, the chances of a quick recovery are higher in comparison to patients receiving a stem cell transplant or extensive chemotherapy (Benmebarek et al., 2019).

Living Drug: Being a "living drug" is the greatest amazing aspect of CAR T-cell treatment. Staying in the Patient body, the genetically engineered T-cells can keep an eye on the cancer

cells and destroy any cancer cells that come back. The therapy's long-term stability may help avoid the need for repetitive treatments (Schietinger & Greenberg, 2024).

Personalized Medicine: CAR-T cell therapy reflects the essential nature of personalized medicine. Indeed, this therapy involves the development of treatment for a specific person's cancer, which makes it more effective for the treatment and reduces the chance of adverse reactions (Shen et al., 2019).

The benefits of CAR T-cell therapy support its transformative effect on cancer treatment, with patients and healthcare practitioners gaining a new dimension of hope in fighting this multifaceted illness.

3.2 Limitations of CAR-T therapy

CAR T-cell therapy is a new paradigm in the treatment of particular cancers, which are mainly indicated for haematological malignancies. The perspective provided by this treatment is viewed as remarkable, but only several nowadays limitations prevent its broader application and success.

Target Antigen Limitations: The first problem that CAR T-cell therapy encounters is the choice of proper target antigens. While CD19 has been demonstrated to be optimum for hematologic malignancies like B-cell leukemias and lymphomas, antigens that share the same degree of familiarity as well as specificity in solid tumours are not typical. The inability to generate targetable antigens on solid tumours constrains the scope of the application in solid tumours (Li et al., 2017).

Tumour Microenvironment: Another important obstacle is the tumour microenvironment. Solid tumours form an immunosuppressive environment that can stop or minimize CAR T-cell function and proliferation. The targets or the physical blockages within the TME, including

stromal cells and extracellular matrix, may also prevent T cells from adequately penetrating and infiltrating the tumour (Schepisi et al., 2020).

Toxicity and side effects: The CAR T-cell therapy causes serious, even fatal, side effects. The two most prevalent and serious adverse effects are cytokine release syndrome and neurotoxicity. Rapid and extensive cytokine release from enabled CAR T-cells causes CRS, which manifests as low blood pressure, high fever, and malfunction in multiple organs. Cerebral edoema, convulsions, and disorientation are some of the signs of neurotoxicity (Mount et al., 2018).

Persistence and Relapse: Although CAR T-cells can help attain remission in a majority of cases, the type of response is not uniformly durable. Unfortunately, many patients tend to experience relapse after CAR T-cell therapy. Relapse is predominantly caused by target antigen loss or mutual exclusive reduced antigen expression; this is also referred to as continuous antigen escape. Moreover, CAR T-cells, like other immunotherapies, are not frequently detected in the patient long after discovery. Hence, this limits their efficiency (Roex et al., 2020).

Manufacturing and Accessibility: Due to the difficulty and resource-intensiveness of the technique, creating CAR T-cells demands specialized facilities and personnel. As a result, it is very costly and the number of patients who can get advantage from the treatment is much lower than it could be otherwise. Additionally, how this treatment is created does not allow it to be mass-produced due to narrow personalization. Thus, it takes more time and resources to produce the necessary dosage (Rurik et al., 2022).

Chapter 4

Current Status

4.1 FDA approved CAR T cell therapies

The Food and Drug Administration (FDA) approved the CAR T cells after successful pre-clinical and clinical trials. The first approved CAR T cell was Kymriah on August 30, 2017. The FDA was able to approve the CAR T-cell therapy because of its success in clinical treatments. This kind of treatment is individualized and reconditions the body's natural defensive mechanisms to act exclusively on cancerous cells. For example, Kymriah helped children who were diagnosed with ALL relapse achieve complete remission from cancer. Indeed, such therapies are not cheap, but they assist the immune system to fight various malignancies thus giving hope. Table 1 shows the list of CAR-T cell therapies approved by the U.S. FDA for different types of malignancies from January 2017 to June 2024.

Table 1: List of CAR-T Cell Therapy for Cancer approved by the FDA

Sl.	Product name	Approval Year	Indication
1.	ABECMA (idecabtagene vicleucel)	2021	Adult patients with relapsed or refractory multiple myeloma.
2.	BREYANZI (lisocabtagene maraleucel)	2021	Adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B cell lymphoma (DLBCL) not otherwise specified (including DLBCL arising from indolent lymphoma), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma and follicular lymphoma grade 3B.
3.	CARVYKTI (ciltacabtagene autoleucel)	2022	Multiple myeloma relapsed and/or refractory after at least 4 cycles of prior therapeutic modalities.
4.	KYMRIAH (tisagenlecleucel)	2017	For adults with relapsed or refractory DLBCL and for young adult patients up to age 25 with relapsed or refractory ALL.

5.	TECARTUS (brexucabtagene autoleucel)	2020	In adult patients who have relapsed or fail to respond to initial therapies for B-cell precursor acute lymphoblastic leukemia. Used in adult patients with mantle cell lymphoma that has relapsed after first line of treatment or fail to respond to initial therapy.
6.	YESCARTA (axicabtagene ciloleucel)	2017	As a third-line treatment in limited populations for adult patients with bulky DLBCL that is either primary refractory to first-line chemoimmunotherapy or relapsed within 12 months of first-line chemoimmunotherapy.

Source: https://www.fda.gov/

4.2 Ongoing Clinical Trials

Currently, several CAR-T cell clinical trials are ongoing. Lymphomas and leukemias are among some of the blood cancers that have been treated effectively using CAR T-cell therapies. CAR-T therapy has mainly shown promising results in treating blood cancers but as for now, there are ongoing trials of solid tumor too. Some of the included types of cancer are glioblastoma, lung cancer, liver cancer, stomach cancer, renal cancer, prostate cancer, osteosarcoma, and others. For example, in March 2024, scientists got an effective outcome on brain tumor by using CAR T cells. Table 2 shows the list of current CAR-T clinical trials for hematological malignancies and table 3 shows the list of current CAR-T clinical trials for solid tumors.

Table 2: Current CAR-T Clinical Trials for Hematological Malignancies

Sl.	**ClinicalTrials.gov Identifier**	**Target Strategy**	**Target Disease**	**Status**
1.	NCT02445248	CD19-specific	Relapsed or refractory DLBCL	Completed
2.	NCT02601313	CD19-specific	Relapsed/refractory mantle cell lymphoma	Active
3.	NCT02348216	CD19-specific	DLBCL, primary mediastinal (internal medicine) common mediastinal large B cell lymphoma (PMBCL), transformation follicular lymphoma (TFL), high-grade B-cell lymphoma (HGBCL)	Completed
4.	NCT02631044	CD19-specific	NHL, DLBCL, follicular lymphoma, mantle-cell lymphoma, PMBCL	Active

5.	NCT02926833	CD19-specific	DLBCL	Completed
6.	NCT02614066	CD19-specific	Relapsed/refractory B-precursor acute lymphoblastic leukemia	Active
7.	NCT03105336	CD19-specific	Refractory/relapse large B cell lymphoma	Active
8.	NCT03287817	CD19- and CD22 specific	DLBCL	Active
9.	NCT03310619	CD19-specific	Aggressive B-NHL	Completed
10.	NCT03568461	CD19-specific	Refractory follicular lymphoma	Active

Table 3:Current CAR-T Clinical Trials for Solid Tumors

Sl.	**ClinicalTrials.gov identifier**	**Target Strategy**	**Target Disease**	**Status**
1.	NCT00004178	CEA CAR	Adenocarcinoma	Completed
2.	NCT00019136	Folate receptor CAR ± IL-2	Ovarian cancer	Completed
3.	NCT00085930	GD2 CAR, EBV T cells	Neuroblastoma	Active
4.	NCT0064196	PSMA CAR	Prostate cancer	Completed
5.	NCT03170141	EGFRvIlI-specific CAR-T cells producing PD-1 and PD-L1 antibodies	Glioblastoma multiforme	Active
6.	NCT00730613	IL-13Ra2 targeting CAR-T cells	Glioblastoma with Hy/TK suicide switch	Completed
7.	NCT00889954	Her2 CAR, EBV T cells + TGFb DNR	Her2 + lung cancer	Completed
8.	NCT02414269	Meso-CART cells, modified with iCasp9/M284	Malignant pleural disease	Active
9.	NCT00902044	Her2 CD28	Her2 + sarcoma	Active
10.	NCT01822652	GD-2-CAR-T with iCas-pase9 suicide safety switch	Neuroblastoma	Active
11.	NCT01460901	GD2 CAR multivirus-specific	Post-allo HSCT neuroblastoma	Completed
12.	NCT01140373	PSMA CAR 2nd	Castrate metastatic	Active
13.	NCT01373047	CEA CAR	CEA + liver metastases	Completed
14.	NCT01454596	EGFRvIII CAR 3rd 28 and 4-1BB ± IL-2	Glioblastoma	Completed

Source for Table 2 and Table 3: https://clinicaltrials.gov/

Chapter 5

Future Implications

Chimeric Antigen Receptor treatment is anticipated to change the field of oncology profoundly and eliminate all types of malignancies. CAR T therapy has seen triumph in treating some kinds of blood cancers and providing hope for patients with no other options. The future will inevitably mean expansion into the majority of solid tumours, which are more complex due to their microenvironment and difficulty in identifying universal target antigens. Nevertheless, the future of this therapy is likely to bring an audience of 1.6 billion viewers and billions in revenue within the next decade (Roex et al., 2020). First off, major innovations in cell engineering, including methods of genetic engineering, will enable the production of cells that are not only efficient but also more resilient and capable of overcoming the tumour's microenvironment (Ramos & Gianpietro Dotti, 2011). Multi-targeting CAR T-cells and so-called 'armored' CAR T-cells, enhanced with additional genes to make them more effective in their function, are currently in development. These changes are aimed at increasing the efficacy and the duration of the T-cell response.

Secondly, the future will likely see the switch from patient-specific autologous CAR T-cells to allogeneic products. Allogeneic CAR T-cells can be made from healthy donors in large amounts and at a lower cost, making it possible to provide a cost-effective and widely available treatment option (Shen et al., 2019).

In the future, with the development of the field, methods of managing side effects will emerge, especially those referring to cytokine release syndrome or neurotoxicity. In the future, the CAR T cells may be engineered to include certain safety clocks which enable doctors to regulate and/or essentially turn off the CAR T cells. This will increase the safety and personal perceptions of the therapy among patients and clinicians (Roex, et al., 2020).

The current manner of cultural manufacture of CAR T cells has flaws and must take additional time to meet the demand. Pharmaceutical companies are currently developing ways to fast-forward the generation process or in other words cut down the time it takes to generate CAR T cells to several days from weeks. This will enhance the delivery log and may also lower the cost as well compared to direct presentation from the author. However, as the therapy progresses, oversight measures will be relevant to enforce formal boundaries on the safe and ethical use of CAR T therapy. The novel nature of these therapies will require discussions and deliberations between scientists, regulatory and licensing authorities, clinicians, and patients to guide the ethical use of CAR T cells (Roybal et al., 2016).

The future of this therapy is extensive and promising in the field of oncology. In the years to come, CAR T cells will be highly efficient, safer, and more common. The options and opportunities for curing previously incurable cancers are almost boundless. CAR T therapy is at the forefront of this medical revolution. Moving forward, the future of the therapy virtually rewrites the patient experience, giving much hope to those infected with cancer worldwide (Rawla, 2019).

The breakthroughs in CAR T therapy are not just another step in the development of cancer treatments; they are a new stage in our fight against the disease. Every discovery brings humanity one step closer to the day when cancer is no longer a deadly diagnosis but a manageable condition and, maybe, one day, a treatable one.

References

ClinicalTrials.gov. (2023). Available at: https://clinicaltrials.gov/

U.S. Food and Drug Administration. (2024) Available at: https://www.fda.gov/

Li, S., Natnaree Siriwon, Zhang, X., Yang, S., Jin, T., He, F., … Wang, P. (2017). Enhanced Cancer Immunotherapy by Chimeric Antigen Receptor–Modified T Cells Engineered to Secrete Checkpoint Inhibitors. *Clinical Cancer Research*, *23*(22), 6982–6992. https://doi.org/10.1158/1078-0432.ccr-17-0867

Mohamed Reda Benmebarek, Clara Helke Karches, Bruno Loureiro Cadilha, Lesch, S., Endres, S., & Kobold, S. (2019). Killing Mechanisms of Chimeric Antigen Receptor (CAR) T Cells. *International Journal of Molecular Sciences*, *20*(6), 1283–1283. https://doi.org/10.3390/ijms20061283

Mount, C. W., Majzner, R. G., Shree Sundaresh, Arnold, E. P., Meena Kadapakkam, Haile, S., … Mackall, C. L. (2018). Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M+ diffuse midline gliomas. *Nature Medicine*, *24*(5), 572–579. https://doi.org/10.1038/s41591-018-0006-x

Morello, A., Sadelain, M., & Adusumilli, P. S. (2016). Mesothelin-Targeted CARs: Driving T Cells to Solid Tumors. *Cancer Discovery*, *6*(2), 133–146. https://doi.org/10.1158/2159-8290.cd-15-0583

Nagata, S., & Tanaka, M. (2017). Programmed cell death and the immune system. *Nature Reviews. Immunology*, *17*(5), 333–340. https://doi.org/10.1038/nri.2016.153

Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., Miklos, D. B., Jacobson, C. A., … McSweeney, P. (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-

Cell Lymphoma. *New England Journal of Medicine/˜ the œNew England Journal of Medicine*, *377*(26), 2531–2544. https://doi.org/10.1056/nejmoa1707447

Pehlivan, K. C., Duncan, B. B., & Lee, D. W. (2018). CAR-T Cell Therapy for Acute Lymphoblastic Leukemia: Transforming the Treatment of Relapsed and Refractory Disease. *Current Hematologic Malignancy Reports*, *13*(5), 396–406. https://doi.org/10.1007/s11899-018-0470-x

Pinz, K., Liu, H., Golightly, M., Jares, A., Lan, F., Zieve, G. W., … Ma, Y. (2015). Preclinical targeting of human T-cell malignancies using CD4-specific chimeric antigen receptor (CAR)-engineered T cells. *Leukemia*, *30*(3), 701–707. https://doi.org/10.1038/leu.2015.311

Porter, D. L., Hwang, W.-T., Frey, N. V., Lacey, S. F., Shaw, P. A., Loren, A. W., … June, C. H. (2015). Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Science Translational Medicine*, *7*(303). https://doi.org/10.1126/scitranslmed.aac5415

Porter, D. L., Levine, B. L., Kalos, M., Bagg, A., & June, C. H. (2021). Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia. *New England Journal of Medicine/the New England Journal of Medicine*, *365*(8), 725–733. https://doi.org/10.1056/nejmoa1103849

Ramos, C. A., & Gianpietro Dotti. (2011). Chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy. *Expert Opinion on Biological Therapy*, *11*(7), 855–873. https://doi.org/10.1517/14712598.2011.573476

Rawla. P., (2019). Epidemiology of Prostate Cancer. *World Journal of Oncology*, *10*(2), 63–89. https://doi.org/10.14740/wjon1191

Roybal, K. T., Williams, J. Z., Morsut, L., Rupp, L. J., Kolinko, I., Choe, J. H., … Lim, W. A. (2016). Engineering T Cells with Customized Therapeutic Response Programs Using Synthetic Notch Receptors. *Cell*, *167*(2), 419-432.e16. https://doi.org/10.1016/j.cell.2016.09.011

Ritchie, D. S., Neeson, P. J., Amit Khot, Peinert, S., Tai, T., Tainton, K., … Darcy, P. K. (2013). Persistence and Efficacy of Second Generation CAR T Cell Against the LeY Antigen in Acute Myeloid Leukemia. *Molecular Therapy*, *21*(11), 2122–2129. https://doi.org/10.1038/mt.2013.154

Roex, Timmers, M., Wouters, K., Campillo-Davo, D., Flumens, D., Wilfried Schroyens, … Sébastien Anguille. (2020). Safety and clinical efficacy of BCMA CAR-T-cell therapy in multiple myeloma. *Journal of Hematology & Oncology*, *13*(1). https://doi.org/10.1186/s13045-020-01001-1

Rurik, J. G., István Tombácz, Amir Yadegari, Méndez, P. O., Shewale, S. V., Li, L., … Epstein, J. A. (2022). CAR T cells produced in vivo to treat cardiac injury. *Science*, *375*(6576), 91–96. https://doi.org/10.1126/science.abm0594

Singh, R., Shaik, S., Bhupender Singh Negi, Jagadish Prasad Rajguru, Pankaj Bajirao Patil, Anuj Singh Parihar, & Sharma, U. (2023). Non-Hodgkin's lymphoma: A review. *Journal of Family Medicine and Primary Care*, *9*(4), 1834–1834. https://doi.org/10.4103/jfmpc.jfmpc_1037_19

Schietinger, A., & Greenberg, P. D. (2024). Tolerance and exhaustion: defining mechanisms of T cell dysfunction. *Trends in Immunology*, *35*(2), 51–60. https://doi.org/10.1016/j.it.2013.10.001

Schepisi, G., Conteduca, V., Casadei, C., Giorgia Gurioli, Rossi, L., Gallà, V., … Ugo De Giorgi. (2020). Potential Application of Chimeric Antigen Receptor (CAR)-T Cell Therapy in Renal Cell Tumors. *Frontiers in Oncology*, *10*. https://doi.org/10.3389/fonc.2020.565857

Shen, L., Li, H., Bin, S., Li, P., Chen, J., Gu, H., & Yuan, W. (2019). The efficacy of third generation anti-HER2 chimeric antigen receptor T-cells in combination with PD1 blockade against malignant glioblastoma cells. *Oncology Reports*. https://doi.org/10.3892/or.2019.7263

Tchou, J., Zhao, Y., Levine, B. L., Zhang, P. J., Davis, M. M., Jan Joseph Melenhorst, … Matro, J. M. (2017). Safety and Efficacy of Intratumoral Injections of Chimeric Antigen Receptor (CAR) T Cells in Metastatic Breast Cancer. *Cancer Immunology Research*, *5*(12), 1152–1161. https://doi.org/10.1158/2326-6066.cir-17-0189

Watanabe, A., Miyake, K., Koshi Akahane, Kumiko Goi, Kagami, K., Hideo Yagita, & Takeshi Inukai. (2021). Epigenetic Modification of Death Receptor Genes for TRAIL and TRAIL Resistance in Childhood B-Cell Precursor Acute Lymphoblastic Leukemia. *Genes*, *12*(6), 864–864. https://doi.org/10.3390/genes12060864