IMMUNOTHERAPEUTIC APPROACHES TO TREAT CANCER: PROSPECTS AND CHALLENGES

By

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Declaration

It is hereby declared that

1. The thesis submitted is my 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.
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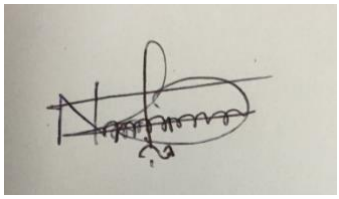

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Approval

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Ethics Statement

This study does not involve any human or animal trial.

Abstract

This review examines and summarizes major novel immunotherapeutic modalities used in cancer immunotherapy that are currently approved or in clinical trials, as well as their therapeutic potential, side effects, challenges, and future directions The aim of this study was to show different immunotherapeutic modalities used to treat cancer. The mechanism of action of the immune system to combat cancer e.g. Adoptive T cell transfer therapy (TIL, TCR, CAR T Cells), Immune checkpoint inhibitors, monoclonal antibodies cancer vaccines, cytokines and enzyme therapeutic. Second, the study aims to create awareness and provide hope to cancer patients as well as to academics interested in learning more about immunotherapy. Immunotherapy has shown great potential and promise in the treatment of a variety of cancers like solid tumors and hematologic malignancies. Finally, it discusses immunotherapy treatment prospects in the future, including combination therapy.

Keywords: Immunotherapy; TIL, TCR; CAR T Cells; Monoclonal antibodies, Checkpoint inhibitors

Dedication

Dedicated to my teachers, family and friends

Acknowledgement

I am eternally grateful to the Almighty God for blessing me with good health, courage, and wisdom, allowing me to successfully complete my undergraduate thesis.

I want to thank my Parents, Mrs Nadunga Rofina, and Mr Wonambwa David, my siblings Namugowa Ronald, Namugowa James, Namugowa Zephania, Birungi Sarah,Muyama Norah and Mulokwa Michael for their support and words of encouragement during the most difficult times .

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List of Acronyms

ACT	Adoptive Transfer T-Cell Therapy
TIL	Tumor Infiltration Lymphocytes
TCR	T Cell Receptor
CAR T cell	Chimeric Antigen Receptors
NK	Natural Killer
CD	Cluster Differentiation
IFNγ	Interferon Gamma
IL-2	Interleukin -2
TAA	Tumor Associated Antigen
MHC	Major Histocompatibility Complex
NY-ESO-1	New York esophageal Squamous cell Carcinoma -1
MART1	Melanoma Antigen Recognized by T cell- 1
HLA	Human Leukocyte Antigen
ALL	Acute lymphoblastic Leukemia
CR	Complete Response
DLBCL	Diffuse Large B cell Lymphoma
FDA	Food and Drug Administration
APC	Antigen Presenting Cell
P53	Tumor suppressor Protein
MAGE	Melanoma Antigen Gene

Chapter 1

Introduction

Cancer is a disease with unique properties. It is characterized by uncontrolled proliferation of cells and is often accompanied by metastasis as it infiltrates normal cells and tissues (Y. Zhang & Zhang, 2020).The disease is suggested to be caused by several factors like body aging cells, smoking, a mutation in tumor suppressor genes and proto-onco as a result of its inability to work, exposure of the body to a non-ionizing electromagnetic field, and lastly uncontrolled cell proliferation (López-lázaro, 2016). The genomic variation produced as a result of mutation may produce tumor neoantigen thus triggering a response of the immune system (Y. Zhang & Zhang, 2020). The conventional modes of treatment for cancer patients previously used include radiotherapy, chemotherapy, surgery, and target therapy. Over the last decade, immunotherapy has emerged as a very promising approach for cancer treatment because of its unique way of dealing with and eliminating different cancer cells by indirectly boosting the host's immune system to fight cancer. However, cancer cells are complicated cells that employ specialised, unique characteristics like metastasis and uncontrolled growth compared to normal cells for their growth and development. Research has shown that cancer disease has got eight hallmarks that could enable it to escape immune surveillance. These include evading growth suppressors, enhancing replicative immortality, maintaining proliferative signaling, resisting cell apoptosis, inducing angiogenesis, evading immune destruction, activating metastasis and energy metabolism reprogramming takeover (Zhou, 2014). The data published by the World Health Organization's International Agency for Cancer Research (IARC) indicates that cancer numbers have risen to 19.30 million new patients with 10 million cancer deaths worldwide in 2020. Cancer is perceived as a global threat to humanity, with a projected burden estimated to be approximately 28.40 million new

cases, a 47% increase from the 19.30 million cases in 2020. The common types of cancer account for at least 60% of the newly diagnosed cancer cases and more than 70% cancer deaths in 2020.18 % of the total cancer deaths lung cancer making it the leading cause of cancer deaths followed by cancers of the colon and rectum, liver cancer, stomach cancer and breast cancer according to (Sung et al., 2021). This exponential increase in new cancer cases has prompted researchers worldwide to develop a proper effective treatment that could be a solution to this global health problem, pushing them to explore the concept of immune system in treatment of diseases. Precursors to immunotherapy can be traced back to the late nineteenth century, when Dr. William B. Coley (Dine et al., 2017), a working bone surgeon in New York, injected bacteria to treat osteosarcoma. This was inspired by one of his patients, a young lady suffering from hand osteosarcoma, a type of cancer that affects the bones in the hands. Despite his surgical efforts for arm amputation, she died of metastatic disease within months of the operation. This prompted Coley to learn more about the disease from which she was suffering, and he began reviewing all of the hospital's medical records from ninety sarcoma patients. During the examination, he was surprised to learn that a patient diagnosed with a sarcoma who could not be operated on had complete tumor regression after developing erysipelas, a type of skin infection. Coley was taken aback, and he wondered if cancer patients injected with erysipelas could be cured. He obtained Streptococcus pyogenes, which causes erysipelas, and began inoculating Streptococcus pyogenes toxins into sarcoma patients using live bacterials. Many of his patients died, so he decided to use a mixture of heat-killed Streptococcus pyogenes and Serratia marcescens. His findings were especially intriguing because he discovered outstanding success with his toxins, which induced patient tumor regression. He wrote literature concluding that toxins are capable of inducing tumor regression. Coley had no idea how the toxins worked, but he remarkably compiled great evidence connecting cancer and the immune system, earning him the title "Father of

Immunotherapy" despite his findings being highly controversial and harshly criticized (Carlson et al., 2020).

The body uses an immunosurveillance mechanism to detect foreign substances. Immune surveillance, according to Paul Ehrlich, was present, and it involved removing nascent transformed cells until they were clinically visible. While Burnet and Thomas expanded on this theory and proposed an immune surveillance theory supported by experimental evidence, they hypothesized that the regulation of emerging transformed cells comprises an old immune system that plays an important role in surveillance. The results of the experiment revealed that transplanted tumors in mice were rejected due to a strong immune-mediated system (Kim et al., 2007). Immune surveillance, it has been stated, primarily serves as a component of a larger process known as immune editing, which consists of three major stages. The concept of immunoediting is attributed to Schreiber and Dunn who talks about cancer development stages. The first phase is the elimination; here, the potential tumor cells develop but do not progress to clinical status due to the release of highly immunogenic immune cells such as CD4+, CD8+ and NK cells, which recognize neoantigens from tumor cells to kill and eliminate them. The second Equilibrium Phase is the phase in which there is a balance between tumor cell death caused by the immune system and tumor cell proliferation because the immune system produces less immunogenic cells that are unable to completely destroy tumors however, they still remain subclinically inactive and dominant. The third is the phase of escape. It denotes the tumor's stage of development in which the tumor variant evades the immune system and becomes clinically detectable as a cancer cell (Ribatti, 2017)**.**

Scientists have since then advanced on the concept of exploiting the immune system of the host in developing a novel strategy of treatment for cancer. The immune system is indirectly used to eradicate and overcome cancer as it employs immune surveillance mechanisms to

defend the body from foreign substances. Cancer immunotherapy is a novel approach of treatment that uses the patient's immune system to fight cancer. Immune-oncology has shown positive prospects as one of the immunotherapeutic fields that are advancing clinically by enhancing the inherent abilities of the body's immunity to control the growth of tumors. With all the excitement and attention cancer immunotherapy has gained, it is considered a game changer and could be the solution to cancer treatment by harnessing the host immune system to potentially recognize the specific targeted abnormal cancer cells. Cancer immunotherapy, for example, is used to treat cancers that have become resistant to previous treatments. Therefore, with the advanced technology, several promising new strategies in the field of immunotherapeutics in immuno-oncology are emerging and these modalities include immune checkpoint inhibitors, adaptive transfer cell therapy, cytokines, and monoclonal antibody (Farkona et al., 2016). Enzyme immunotherapeutic therapy and cancer vaccines have also shown some promise. In this review, we discuss and summarize major novel immunotherapeutic modalities that are currently approved or in clinical trials, as well as their therapeutic potential, side effects, challenges, and future directions.

Chapter 2

Methods

Extensive literature review on immunotherapy in cancer treatment using secondary research methods such as research articles, news articles, academic published papers, and government web sites, Cancer.Net website. Articles from distinguished journals such as, Nature, Elsevier and MDPI were analysed for this study. In addition to this method, qualitative method was used to conduct this review. Data was collected from various journals with their findings, which assisted in the identification of variable clinical data that could play a significant role in the future aspects of this cancer immunotherapy, such as combination therapy.

Chapter 3

Immunotherapeutic Strategies

3.1 Immune-enhancers

Immune enhancers are substances that act as boosters in enhancing the immune system by potentiating its activity by increasing the immune response. This results in protecting the body against invading foreign substances like infections or genetic mutations that could arise during cellular replication and trigger neoantigen development. The immune system's activity can be harnessed or enhanced by using genetically engineered molecules similar to components present in the immune system. Adoptive cell transfer therapy is one of them. Monoclonal antibodies, checkpoint inhibitors, cytokines, and enzyme immunotherapeutics are examples of immune enhancers. Lastly cancer vaccines with live attenuated or dead bacteria, virus’s adjuvants like inactive toxins (toxoids) could cause immune response by enhancing the body's immune system to produce antibodies for defense against a specific pathogen in the body (Marcia Zimmerman, C.N., 1999).

3.2 Adoptive Transfer T-Cell Therapy (ACT)

ACT is an immunotherapeutic approach that utilizes several modalities to potentiate the immune response of cellular mediated immunity by enhancing and boosting the immune system's ability to identify and eliminate tumor neoantigens of cancer. This therapy comprises of tumor-infiltrating lymphocyte (TIL) which is derived from T-lymphocytes obtained from a patient having a specific type of tumor. They are expanded and reintroduced back in the patient. Genetically modified T cells (MIT (Massachusetts Institute of Technology), 2020), like TCR T Cell and CAR T Cells. These are targeted cellular therapies that eradicate tumor cells by using the cytotoxic potential of the T cells in an antigen-specific manner as they express tumors recognizing receptors that can cause stimulatory signaling effects of the T-cells during its activation. Several Adoptive Cell Transfer modalities are undergoing preclinical and clinical testing. Some T-cell therapies generated have already been approved by the FDA to treat cancer patients having B cell malignancies (Met et al., 2019) .The process of production of Adoptive cell Transfer cell therapy modalities except TIL therapy involves taking blood from a patient with a specific type of tumor neoantigen, autologous T cell isolation to obtain the required T cells. The isolated T cells are then engineered, expanded in the lab, and activated with the help of IL-2. Then they are reintroduced back into the patient by infusion. For example, autologous T cells are genetically modified so that they can express the desired new TCR and CAR gene encoding specific T Cell Receptors on the cell surface with a specific antibody that has affinity for a specific antigen (MIT (Massachusetts Institute of Technology), 2020).This therapy has demonstrated positive clinical responses in treatment of several tumor neoantigen for different cancers, for example, melanoma, multiple myeloma and metastatic synovial sarcoma (Hammerl et al., 2018). The rapid advances in technology has accelerated research in immunotherapy field of immuno-oncology and it has expanded the knowledge of cellular

and molecular understanding on how immune cells when modified genetically can contribute to a reduction in numbers and activation of intra-tumor T cells and it demonstrated great therapeutic potential in cancer treatment (Kunert & Debets, 2018).

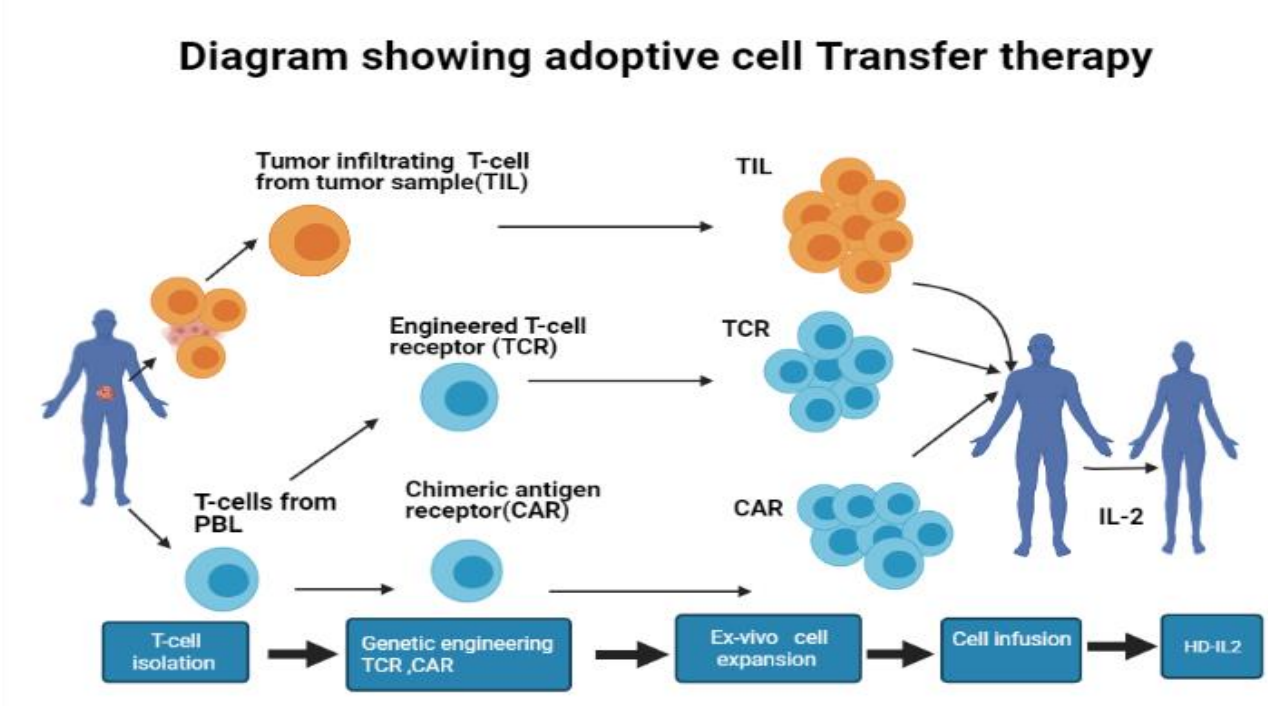

Figure 1: A schematic diagram of adoptive cell therapy (adapted from Wolf et al., 2019)

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3.2.1 Tumor Infiltrating Lymphocyte Therapy

By using Lymphocytes that infiltrate tumors, also known as TIL, treatment in cancer patients, it's considered the first line treatment. It is the most preferred type of treatment to patients during their initial treatment of tumors compared to new ACTs that have been established and developed. Thus, TIL therapy is not taken as an alternative in the treatment of cancer. The discovery of 1986 illustrated that the TIL that expanded in IL-2 could produce TIL cells which had antitumor properties in unimmunized mice. This was presented as a great discovery that opened several clinical trials in humans at the National Cancer Institute in

2002 where TIL and IL-2 were used in patients with metastatic melanoma. The idea was to first decrease the T cells present to allow for the new infused TIL cells to survive in the body. This was made sure by giving lymph depleting agents which include two chemotherapy drugs e.g fludarabine with cyclophosphamide before the therapy was infused in the patient's body. This gave a 50 % response rate. TIL therapy is said to possess the longest clinical experiences as it has specifically displayed a consistent efficacy for cancers like metastatic melanoma, especially cancers in stage (III-IV). However, Tumor Infiltrating Lymphocyte therapy has now advanced to be used in several solid tumors like cancer of the kidney, lungs, gastric, cervix and Head and Neck cancer (Sim et al., 2014). According to Cancer Research Institute & Moffitt Cancer Center, TIL therapy is an example of cellular immunotherapy that utilizes the body's immune system to destroy tumor neoantigens. TILs by definition are a subpopulation of several different lymphocytes, but mainly natural killer cells and T-cells that naturally migrate into the tissue with cancer and reside in the tumor especially in solid malignancies. The Capability of TILs to recognize tumor neoantigens by their endogenous T-cell receptors has favoured their progenesis in several cancers (Met et al., 2019). Tumor Infiltrating Lymphocyte controls tumor growth and development in most solid tumors.

3.2.2 Tumor Infiltrating Lymphocytes Manufacture and Expansion Process

The naturally occurring T cells that have infiltrated the tumor in the patients are collected during surgery or small tissue biopsies and are cut up into 3-5mm^2 fragments from patients with a malignancy (Sim et al., 2014). These autologous T cells are isolated to get the required T cells , allowed to expand for approximately 14 days to 21 days (Stroncek et al., 2019), and then activated in the laboratory ex vivo with a medium change of fresh interleukin -2 which is done frequently to enhance rapid proliferation and expansion and their survival (Sim et al., 2014). After a TIL dose of 720,00IU/kg for every 8h is injected for 4 consecutive days, IL-2 in appropriate doses are given on days 1 and 21 respectively (Sim et al., 2014). The

activated T-lymphocytes can recognize, destroy and eliminate those solid melanoma cancer cells with a short procedure of chemotherapy done before the infusion of TIL (Radvanyi, 2015)

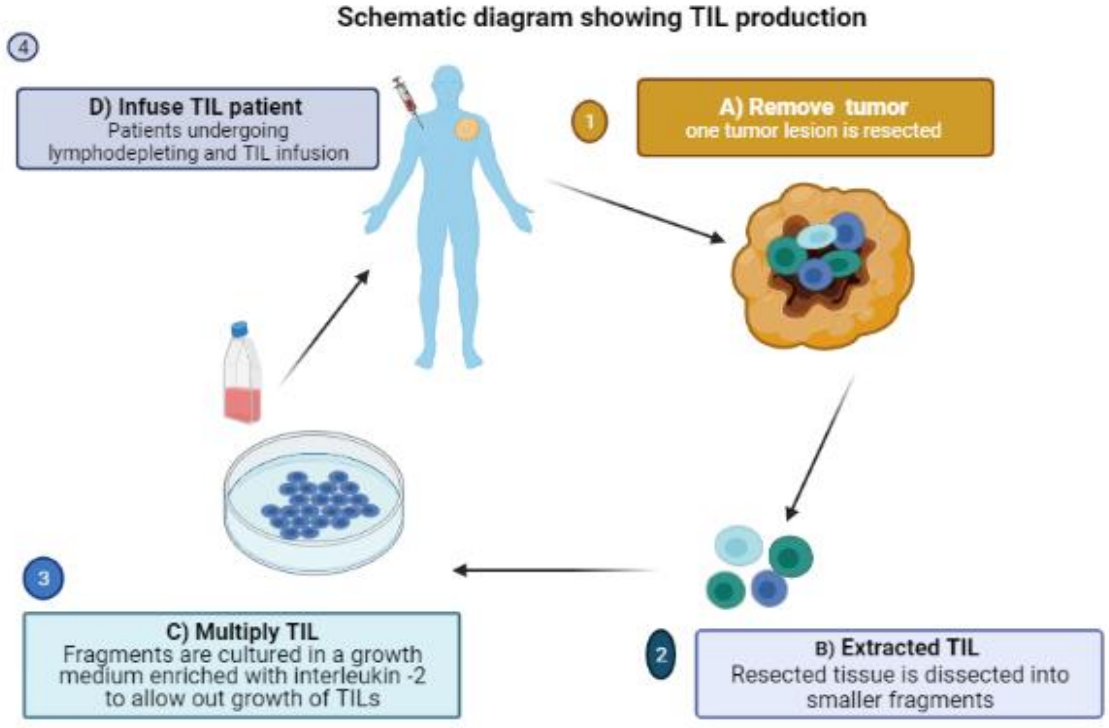

Figure 2: Tumor infiltrating lymphocyte production (adapted from (TILs, the Ultimate in Personalized Immunotherapy, Move Closer to Market, n.d.))

3.2.3 Mechanism of Action of TIL

TIL modality therapy treatment of malignancies use autologous activated T lymphocytes obtained from patient with a tumor (Dafni et al., 2019). The activated autologous Cytotoxic T lymphocytes (CTLs) are CD8+T cells isolated which have the potential to recognize and destroy the malignant tumors. These CTLs immune cells can control tumor malignancy

growth as they differentiate into early T cells with effector memory functions, as well as a number of more CD8+ T-cells with effector functions according to which MHC class associated with the antigen thus contributing more potently to tumor regression during adoptive cell transfer (Sim et al., 2014) .These autologous T-cells kill these malignant tumors by producing cytokines like IFNγ whose role is to cause activation of cellular immunity which lead to stimulation of antitumor immune responses by activating NK cell and macrophages (Maibach et al., 2020).The immune system recognizes the developing malignant tumor cell. Hence, inflammatory signals increase at the site tumor location, triggering the production of Cytotoxic T cells response, and causing tumor destruction (Santoiemma & Powell, 2015).

3.2.4 Current Applications of TIL in Oncology

High dose IL-2 with TIL has already shown 50% rate of response in patients with metastatic melanoma in the most clinical center around the globe. On the other hand, National Cancer Institute clinical trials demonstrated that they have achieved a 51% response rate with a complete and durable regression of 13% in patients with cancer (Sim et al., 2014).TIL therapy in the treatment of cancer has shown a positive clinical response for patients with melanoma, demonstrating that 33%-56% patients who received Tumor Infiltrating Lymphocyte treatment had responded completely to the treatment and 24% to 29% partially responded. The majority showed complete responses for long duration of up to 5 years with 20% of patients having no relapses. TIL therapy is now being explored in the treatment of several solid malignancies such as in the treatment of ovarian, lung, breast, and colon cancers and it has shown positive clinical responses (Stanton & Disis, 2016).

3.2.5 Pharmacodynamics (Specificity, Efficacy and Potency)

Tumor infiltrating lymphocytes therapy has high antigen specificity as it targets specific tumor associated antigens (TAA) only and it has also shown antitumor efficacy by demonstrating a durable long-term response in heavily pretreated patients (Sarnaik et al., 2020). The potency of TIL therapy is said to be effective due to the achieved 50% positive therapeutic response rate and total response rate of 20%. However, this response rate varies from patients to patients (Sim et al., 2014).

3.2.6 Challenges of TIL Therapy

There is a challenge to manufacture a large proportion of TIL T-cells with the required antigen specificity and great efficacy for patients. Also, the need to use billions of cells, at least 40-60 billion cells are needed to infuse into the patients to produce consistent clinical activity (Sim et al., 2014). The remission of the tumor due to loss of durable long-term clinical responses may occur because of loss of proliferative ability and costimulatory molecules for signaling (Wu et al., 2012). Lastly, could possibly be as a result of some patients may have tumors that may not yield TILs that are productive for the therapy (Sim et al., 2014).

3.2.7 Solutions to the Challenges

Predictive biomarkers are used to establish tumors that are able to produce productive TILs for the therapy to avoid working on probability because sometimes not all patients give productive TILs for the TIL therapy (Sim et al., 2014).Remission of tumors with loss of durable long-term clinical responses due to loss of proliferative capacity can be solved by injection with interleukin-2 (IL-2) to enhance rapid continuous T cell proliferation and their survival for a long period (Sim et al., 2014).

3.2.8 Adverse Effects

Toxicity may occur because of administration of cytokines like IL-2 in high doses. This may produce cytokine release syndrome hence can be overcome by use of minimal dose of the IL-2 however, it is still under investigation (Sim et al., 2014).

Table 1: Tumor Infiltrating Lymphocytes therapy approved by FDA (Sarnaik et al., 2020)

Brand Name &(Generic Name) of TIL Therapy	Therapeutic indication	Date of authorization (FDA)
Lifileucel formerly LN-145	advanced cervical cancer	May 2019

3.3 TCR-T Cell Therapy

It is an adoptive cell transfer therapy that involves modification of T cells receptors that express new T cells and could potentially recognize a specific tumor antigen to elicit an immune response. This novel TCR generated has demonstrated efficacy in producing effective immune response by increasing T cell activation through cell signaling to the target of a specific antigen (Stroncek et al., 2019). They use a pair of heterodimeric polypeptide chain, which are specific having both variable regions and constant regions. These T cell receptors possess β, α (beta ,alpha) polypeptide-chains respectively (95%) with a remaining small portion expressing gamma(γ) and delta(δ) (Barritt, 1982),which recognize polypeptide fragments presented by the Major Histocompatibility (MHC). The T cells are collected from patients with cancer .They are then isolated, genetically engineered in the laboratory (ex *vivo*) and expanded. Then it is reintroduced into the patient in order to boost the immune system's response to kill cancer. The TCR therapy was demonstrated by Dr. Michael Steinmertz who was the first to transfer the TCR gene from one T cell and introduced it in the second cell

with the same tumor antigen. The result demonstrated T cell recognition of tumor neoantigen (L. Zhao & Cao, 2019).

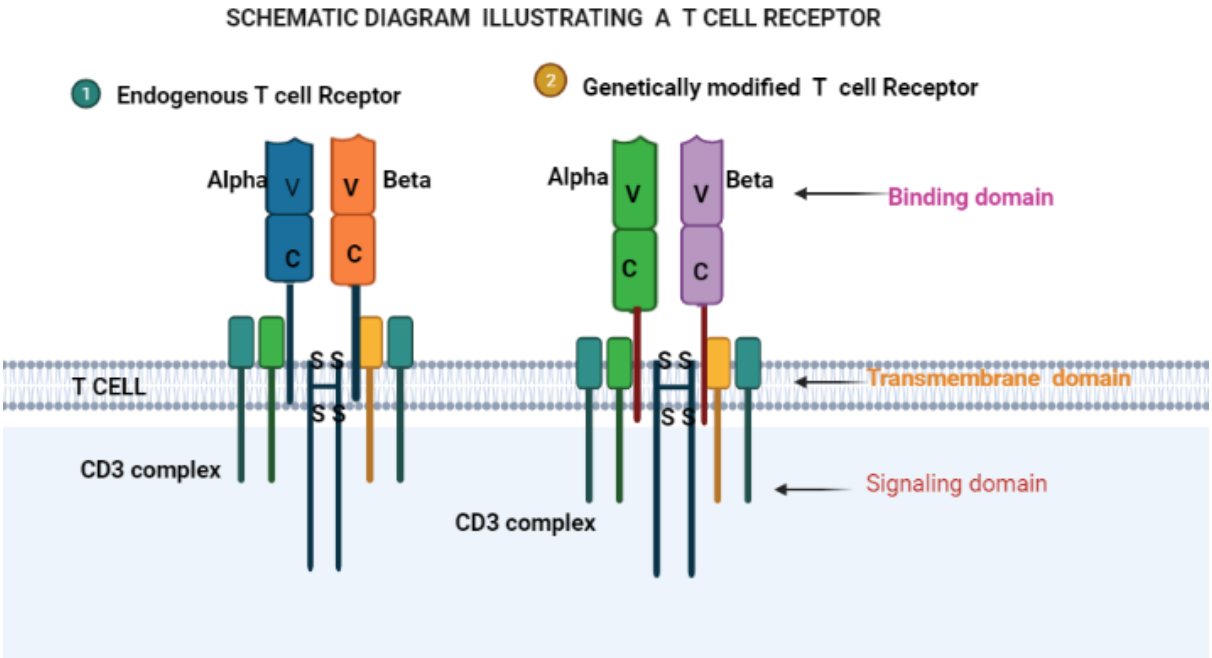

Figure 3: T-cell responses to cancer can be manipulated and redirected by modifying endogenous TCRs, which may increase their affinity and specificity for tumor neoantigens. Genetic engineering: T cells receive gene sequences that codes for new TCRs alpha and beta polypeptide chains with different specificities. To reduce mispairing of the inter-chains with the intracellular T Cell Receptors, modifications such as the addition of a disulphide bridge are used (Sharpe & Mount, 2015).

The process of production of TCR-engineered T cells involves collecting Peripheral Blood Mononuclear Cell (PBMNC) concentrates of autologous T Cells by apheresis, isolating them, and introducing a gene that codes for new receptors on the T cells. Then they are expanded by transducing genes encoding the TCR-alpha and TCR-beta chains. These Autologous T lymphocytes are redirected to detect specific tumor neoantigens. The main purpose of TCR T technology is to modify T lymphocytes to bind to specific tumor antigens because a person's conventional T-Cell receptor affinity for these tumor antigens is low, making it difficult to effectively recognize and kill cancer cells (L. Zhao & Cao, 2019). Zinkernagel and Doherty discovered in 1974 that foreign peptide of fragments present on the MHC proteins could be recognized by T cell receptors and thus T cells with the same MHC alleles activate T cells,

providing a mechanism for immune cells to recognize intracellular proteins via Interactions between TCR peptides and MHC (He, Jiang, et al., 2019). As a result, any antigen presented by MHC molecules on the surface, intracellular, or neoantigen produced by tumor cells is easily recognized by TCR T Cells, giving it a broader target range (L. Zhao & Cao, 2019). TCR-modified T cells recognize antigens via an MHC-dependent mechanism, implying that targetable antigens are tissue-specific antigens (Rohaan et al., 2019).TCR-peptide MHC interactions perform specific immune surveillance functions, such as mediating cell apoptosis through the secretion of cytotoxic granules, cytokines, or perforin. Several studies have made progress in examining the interaction of TCR with peptide- MHC complexes. By using a small number of T cells cultured in a laboratory to generate tumor antigen-specific T cells, they have demonstrated the effectiveness of eradicating tumor cells via tumor antigen-specific T cells (He, Jiang, et al., 2019). More than 84 TCR-T immunotherapy clinical trials are currently registered on the clinictrials.gov website, demonstrating TCR T Cell's potential in cancer immunotherapy (He, Jiang, et al., 2019). There are different ways of categorizing tumor antigens targeted in TCR-T-cell therapy. Human cancer antigens are divided into two types: shared tumor-associated antigens (shared TAAs) and unique tumor-associated antigens (unique TAAs), also known as tumor-specific antigens (J. Zhang & Wang, 2019). TCRs expressed on the surface of T cells recognize and interact with the peptide/MHC complex, resulting in a variety of effects including T cell proliferation and differentiation as well as cytokine or chemokine secretion.

3.3.1 Mechanism of Action of TCR-T Therapy

TCR-T therapy employs heterodimers of two subunits, alpha and beta polypeptide chains, each with three loops known as complementary determining regions (CDR), which allow the T cell to recognize the polypeptide fragments presented by MHC (L. Zhao & Cao,

2019).APCs digest antigens and showcase fragments on major histocompatibility complex (MHC) molecules. As the target peptide sequence is presented by the required major histocompatibility complex class-1 for effective cytotoxic T cell activation, this MHC/antigen complex binds to the TCR and interacts with the antigen present on the target cell (Sharpe & Mount, 2015), with other co-stimulatory molecules such as CD28 activation results in T cell activation, proliferation, differentiation, with cytokine release and apoptosis. T cell function is stimulated after the TCR interacts with short peptides presented by MHC class I or II molecules, such as MHC 1 for CD8 T cells and MHC II for CD4 T cells. However, the TCR heterodimer cannot initiate T cell activation by itself because it lacks the ability to activate downstream pathways because they are short. TCR signaling must be initiated by co-receptors such as CD4 for helper T cells and CD8 for cytotoxic T cells (Barritt, 1982).

3.3.2 Pharmacodynamics (Specificity/Efficacy and Potency)

TCR Therapy is an antigen-specific therapy that uses specific receptor types to target a specific cancer neoantigen. TCR T-Cells with high affinity are created after a specific antigen is identified (L. Zhao & Cao, 2019). For example, T cells engineered to express TCRs specific for NY-ESO-1 have been shown to be safe and feasible in cancer patients (Rath & Arber, 2020), who overexpress such multiple myeloma. TCR therapy is reported to have a high affinity for tumor-associated antigens (TAAs), with clinical trials demonstrating that 80 percent of patients with multiple myeloma have a good clinical response, with approximately 70 percent having a near-complete or complete response (L. Zhao & Cao, 2019). Several factors may influence the efficacy and potency of TCR therapy, including the tumor microenvironment, which is made up of vasculature, tumor cells. It is characterized by an immune-suppressive environment, such as lack of molecules that promote Dendritic Cell

function, which affects antigen presentation and has the potential to result in T-cell activation and T-cell tolerance (Sharpe & Mount, 2015).

3.3.3. TCR–T Cell Therapy in both Solid Tumors and Hematological Malignancies

When it comes to hematological cancers, TCR-T cell therapy has demonstrated that specific targets are ideal for this therapy, particularly in leukemic stem cells. This is regarded as an excellent therapy for treating a malignant myeloid tumor**.** TCR-T cell therapy preclinical data in a mouse model published by Eureka Therapeutics on antibody TCR –T cell (Ab TCR) therapy showed that antibody TCR-T cell therapy has anticancer effects that can be used to treat hematological cancers such as leukemia and lymphoma, known to respond well to CART cell. However, because of its life-threatening side effects such as neurotoxicity and cytokine release syndrome, this is viewed as an alternative. Many TCR-T cell therapy technologies are used in solid tumors, with several of them in clinical trials as endogenous isolated T Cells that are modified and introduced into the human body for T cells to target and identify multiple solid cancers (L. Zhao & Cao, 2019). Several clinical trial reports have demonstrated the great potential of TCR-T cell therapy as a cancer treatment, with data indicating tumor regression in patients. In the first clinical trial involving a melanoma patient, an endogenous T-cell engineered with a TCR directing it to the melanoma antigen to be identified by T cells (MART1), which had been cloned from a TIL isolated from a biopsied melanoma lesion, was used. According to the findings, two of the seventeen patients had partial tumor regression, no significant toxicity, and T cells that had been modified remained in the body for more than a year. Second, in another clinical trial, 31 patients were treated with TCR-T cell therapy, with four patients achieving significant regression of metastatic melanoma (Sharpe & Mount, 2015). Furthermore, T cell TCRs that have been

engineered to express a specific affinity for NY-ESO-1 have been used to treat patients with melanoma and synovial cell sarcoma. In this trial, 11 (55%) of the 20 patients with NY-ESO-11 melanoma had objective clinical responses, with an estimated 5-year overall survival of 33%. In addition, 61 percent of 18 patients with NY-ESO-11 synovial cell sarcoma had objective clinical responses, with an estimated 5-year overall survival rate of 14 percent, demonstrating that this therapy is effective in the treatment of cancer (Stroncek et al., 2019).

3.3.4 Challenges of TCR –T cell Therapy

TCR-T Cell therapy has demonstrated significant potential therapeutic progress in the treatment of various cancers. However, there are still limitations to this treatment. TCR-T cell technology development is difficult due to numerous challenges, such as selecting a good target. For instance, a tumor-specific TCR isolated from a patient with a Human Leukocyte Antigen (HLA-A) type. HLA-A0201 is only effective in patients who have the HLA type HLA-A0201 (Stroncek et al., 2019).The limitations include evaluation of its safety, screening for optimal TCR affinity, the cost of the therapy, time consuming nature and difficulty to develop (L. Zhao & Cao, 2019).The presence of the TCR's peptide target sequence in other proteins creates a risk of false targeting due to increased TCR affinity (Sharpe & Mount, 2015).This therapy only recognizes antigens presented by specific MHC molecules. Therefore, if MHC molecules are mutated, the tumor cell may escape immune surveillance, posing a clinical limitation. Finally, graft versus host disease may occur as a result of induction of harmful recognition of auto-antigens (L. Zhao & Cao, 2019).

3.3.5 Solutions to Challenges TCR -T cell Therapy

The aforementioned challenges are presented by researchers with the hope that finding a solution will be a significant milestone in the success of TCR cell therapy (L. Zhao & Cao,

2019). Use of biomarkers to easily establish the exact targets present, to reduce the possibility of antigens present in vital normal organs from being targeted by modified TCR T cells. Furthermore, immunoglobulin transfer can reduce the risk of on-target off-tumor toxicity by compensating for the depletion of antibody-producing B cells(Sharpe & Mount, 2015). Also, more memory T cells that can recognize the tumor for an extended period of time should be created in order to solve loss of the ability to recognize the tumors. Finally, a suicide gene should be incorporated into TCR T Cells so that when they over proliferate and begin to destroy normal cells, the gene is activated to destroy them (Bertoletti & Tan, 2020).

3.3.6. Side Effects/Toxicity of TCR Therapy

TCR T-Cell Therapy with Genetically Modified TCRs enhanced to recognize MAGE-A3 (EVDPIGHLY) for myeloma and melanoma may result in lethal cardiac toxicity due to off-target reactivity. Second, because of on-target off-tumor reactivity, destruction of normal cells, melanocyte destruction of the skin, eyes, and ear can sometimes result in hearing loss. Finally, diarrhea and fever may occur as a result of on-target off-tumor reactivity and toxicities, as well as cytokine release syndrome (Sharpe & Mount, 2015)

Table 2: Current clinical targets of TCR-T therapy for solid tumors (ClinicalTrials.gov)

Target	Disease	Stage	Phase	NCT number	Country
MAGE	Solid and hematological malignancies;	Enrolling	–	NCT03391791	Canada
	Metastatic renal cancer and melanoma;	Terminated	I/II	NCT01273181	United States
Gp100	Metastatic melanoma;	Completed	II	NCT00923195	United States
MART-1	Skin metastatic melanoma	Completed	I	NCT00091104	United States

	HPV-associated cancer;	Completed	I/II	NCT02280811	United States
NY-ESO-1	Ovarian, fallopian tube, or primary peritoneal cancer;	Recruiting	I	NCT03691376	United States
P53	Metastatic cancer that overexpresses p53	Completed	II	NCT00393029	United States

3.4 CAR T- Cell Therapy

CAR-engineered therapy is an immunotherapeutic approach that involves modifying the DNA of T-lymphocytes in order to produce an artificial T cell receptor with the ability to redirect its specificity**.** It began with a double chain and then a single chain (scFv) chimeric antigen receptor (CAR) (Yee, 2018). In 1987, Zelig Eshhar, an immunologist from Israel's Weizmann Institute of Science's chemical immunology department, created the first chimeric molecule by engineering T cells (“CAR T-Cell Therapy,” n.d.). CARs are genetically engineered receptors that have been modified to give specific properties of immune effector cells by gaining the affinity and specificity of a monoclonal antibody to target specific tumor neoantigens (Styczyński, 2020). CAR is a recombinant receptor with T cell activation and a tumor binding site (L. Zhao & Cao, 2019).The first CARs developed were ineffective in clinical trials and were not approved for use. For more than three decades, there has been significant advancement and development of Chimeric antigen receptors (CARs), which are being investigated by immunology researchers in order to greatly improve and develop more sophisticated CARs, from first, second, and third generation. Second generation CARs are

CARs that have improved antitumor activity of T lymphocyte cells due to apoptosis resistance, improved T Cell proliferation, cytokine secretion, and finally in vivo persistence (Styczyński, 2020). Following the introduction of a proposal for genetically engineered receptors, Steven Rosenberg, M.D., Ph.D., chief of the Surgery Branch at the National Cancer Institute's (NCI's) Center for Cancer Research lab, reported the first successful CART cell treatment in 2010 for an advanced follicular lymphoma ("CAR T-Cell Therapy," n.d.). The race for cancer treatment, on the other hand, thus continued. A dedicated team at the University of Pennsylvania and Children's Hospital in Philadelphia, led by David Porter, Stephan Grupp, and Carl June, administered CART cells to patients with Acute Lymphoblastic Leukemia (ALL) and Chronic Lymphoblastic Leukemia (CLL) in 2012 and 2011, respectively. In 2012, a seven-year-old Emily white head was cured of refractory or relapsed B cells after receiving anti-CD 19 CAR T cells, which was a huge success in cancer treatment (Styczyński, 2020).Two breakthroughs in CAR development were recorded with the addition of costimulatory signaling domains to second-generation CAR constructs to express human lymphocytes with functional CARs for clinical use. Second, studies demonstrating complete responses in patients with refractory Acute Lymphoblastic Leukemia and chronic lymphocytic leukemia using a CD19-directed CAR T cell product paved the way by heavily influencing trials with FDA approval for their use in B cell malignancies. This had represented a significant step forward in cancer treatment (Yee, 2018). CAR-T cells are undergoing advanced clinical trials currently. Some have already been approved for the treatment of CD19+ hematologic malignancies (Gaissmaier et al., 2020). On August 30, 2017, Novartis had become the first pharmaceutical company to receive FDA approval for the Tisagenlecleucel-T as the first CAR T-cell therapy (Kymriah) suspension for intravenous infusion for young adults up to the age of 25 and pediatric patients with B Cell acute lymphoblastic leukemia (BALL). CAR T cell therapy has also shown promising results in

patients with relapsed or refractory diseases which previously had few treatment options, with complete responses ranging from 40 percent to 60 percent in aggressive lymphomas and 60 percent to 80 percent in acute lymphocytic leukemia (Sermer & Brentjens, 2019).These responses, however, differed from patient to patient. In the Novartis study, 68 patients were infused with CAR T cell products, with 63 patients evaluated for efficacy. Within three months of infusion, 83 percent (52 of 63) 95 percent confidence interval, 71 percent to 91 percent of patients who received Kymriah treatment achieved complete remission (CR) and incomplete blood count recovery (*Novartis Receives First Ever FDA Approval for a CAR-T Cell Therapy, Kymriah (TM)(CTL019), for Children and Young Adults with B-Cell ALL That Is Refractory or Has Relapsed at Least Twice*, n.d.). These approved CAR T cells use, CAR constructs consisting of a CD3-derived T cell activation domain, a single chain variable fragment (scFV) antigen recognition domain, and a costimulatory domain such as CD28, 4-1BB, or both .Following a lymphodepleting conditioning regimen, such as fludarabine and cyclophosphamide, the CAR T cells are then infused into patients (Sermer & Brentjens, 2019).

3.4.1 Mechanism of action of CAR T cell

CAR T-cell therapy is a targeted immunotherapy that involves directly transferring DNA encoding the chimeric Antigen receptor into T cells. The novel receptor is produced with the presence of antibody fragments on their surfaces, allowing the newly produced T cells to attach to the tumor and remain in the body for several years to carry out their function .CAR T Cells are then stimulated by the CAR to produce proinflammatory cytokines as they bind with the targeted tumors, triggering an innate immune cell response in which more cytotoxic T cells are produced in large numbers to kill the tumor cells, thereby eliminating cancer (Dai et al., 2016).

3.4.2 CAR T Cells Therapy Manufacturing Process

T cells are obtained by drawing blood from a patient suffering from cancer through an apheresis process, then the blood components are separated leaving the required autologous T cells which are then genetically engineered by introducing DNA into the T Cells in the laboratory to produce CARs on the T-cell surface. The T cells can simultaneously recognize an antigen on tumor cells that have been specifically targeted to activate the T cells. T cells that have been genetically altered are allowed to multiply and expand in number as they are stimulated by cytokines in laboratory culture. This promotes their proliferation and expansion. The CAR T cells are then frozen and delivered to the hospital where the patient is being treated. However, before they are infused into the patient's body, lymphodepletion is performed (L. Zhao & Cao, 2019).

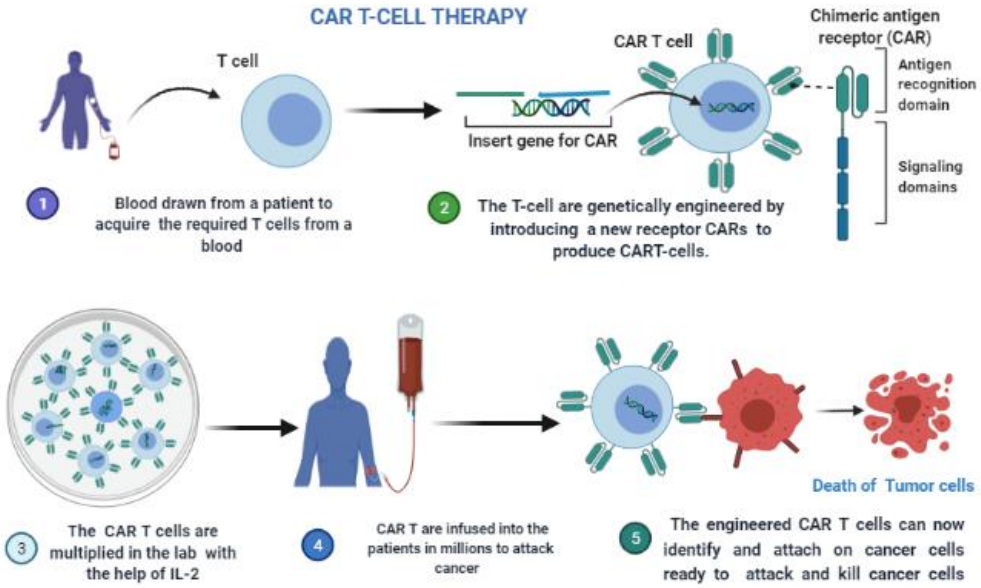

Figure 4. Schematic diagram showing manufacture of CAR T cells (Adapted from American Cancer Society (ACS) website.

3.4.3 Pharmacodynamics (Potency, Efficacy and Specificity)

The first clinical trial of CAR-T-cell therapy in which genetically modified T cells were used in cancer treatment was in 1996 in patients with ovarian cancer. Studies demonstrated limited efficacy (Sharpe & Mount, 2015). According to National Institutes of Health (NIH) research, Novartis and Juno conducted a study that involved Patients with B-ALL who received 4-1BB-based CD19 CAR-T cell therapy achieved a complete Response rate of 83 percent to 93 percent, compared to 70 percent to 88 percent in patients who received CD28 CAR T cell therapy. The information shows that efficacy varies depending on CAR generations. After evaluating the effect of CD28 and 4-1BB expression on CD19 CAR-T treatment in 10 relapse/refractory B-Acute lymphoblastic leukemia patients, according to reports, both types of CAR-T cells stimulated same response rates, but the response was somewhat different, which was due to manufacturing process and infusion dose (X. Zhao et al., 2020). According to Zhao et al., 2020 preclinical and clinical data consistently show and suggest that 4-1BB CAR-T cells are more effective in suppressing B-ALL than CD28 CAR-T cells at low infusion doses. This therapy is a patient specific and customizable approach (personalized therapy) that targets specific cell surface receptors tumor targets like CD19 present on a molecule and is potentially used as a target (Wei et al., 2019). This is because the antigen binding domain can be directed to cancer targets. CAR molecules can target tumor neoantigens expressed on cancer cell surfaces or as part of the MHC complex. Thus, CAR cells make T cell MHC unrestricted, allowing CAR T Cells to recognize a much broader range of targets than natural TCR on T cell surfaces (L. Zhao & Cao, 2019)

3.4.4 CART Cell Therapy Current Application in Oncology

Yescart by Kite Pharma is used to treat adults with relapsed or refractory large B-cell lymphoma and Diffuse Large B cell Lymphoma (DLBCL), while Kymriah by Novartis is

used to treat B-ALL and lymphoma patients. There are two CART Cell therapy that the FDA has approved for the treatment of B cell lymphoma as the CD19 protein (Elahi et al., 2018). According to American Cancer Society, 2020, CART-cell therapy currently approved are Axicabtagene ciloleucel (Yescarta), Brexucabtagene autoleucel (Tecartus), and Tisagenlecleucel (Kymriah). According to Wei et al., 2019, CAR T cell products are used to treat acute lymphoblastic leukemia, chronic lymphoblastic leukemia, and multiple myeloma, which is an incurable disease.

3.4.5 Challenges and Limitations of CAR T cell Therapy

The main difficulty with CAR T cell therapy is that some types of cancers have different antigens which are different from the target ones. Also the presence of the protein antigen targets being present inside the cells rather than on the cell surface, may require CAR T Cells must wear special armor in order to enter the cell and work, posing a challenge. Taking CAR T cells may put the patient at a higher risk of infection because it destroys some of the vital B cells that help fight germs (American Cancer Society, 2020).The therapy is extremely expensive, with Kymriah costing between 510 and 963 US dollars, and Yescarta costing between 402 and 647 US dollars on average (L. Zhao & Cao, 2019).

3.4.6 Side Effects of CART Therapy

Patients on CART cell treatment experience few serious side effects, for example causing a large production of cytokines, a condition known as cytokine release syndrome. These multiply in the patient's body and blood to fight cancer leading to extremely high fevers and dangerously low blood pressure during treatment duration (American Cancer Society, 2020). Neurotoxicity is one of the other side effects. These toxicities were reported following the administration of CAR T cell therapy. One of the patients experienced both acute and

reversible neurotoxicity. B-cell aplasia is another toxicity effect that occurs when anti-CD19 CAR T cells directly destroy B lymphocytes that are normally present in the body and express CD19, exposing patients to a high risk of infection due to hypogammaglobulinemia. Therefore for patients who administered anti-CD19 CAR-T cells have been concurrently infused with intravenous immunoglobulins. Cytokine release syndrome is a potential toxicity that patients may experience following CAR T cell therapy administration which involves massive production of cytokines as a result of CAR T cell recognition of the target. However, CRS toxicity is said to be controlled by the use of corticosteroids and anti-TNF alpha mAb to regulate the effect of excessive cytokine activation (Norelli et al., 2016)

Table 3: FDA Approved CAR-T/TCR Therapies

Brand Name &(Generic Name) of CART Therapy	Therapeutic indication	Date of authorization (FDA)	Patient category
Yescarta (Axicabtagene ciloleucel)	Large B Cell lymphomas	18, October2017	Adult 18
Kymriah (Tisagenlecleucel)	Large B-cell lymphomas	1, May 2018	Adult
	B-cell precursor acute lymphoblastic leukemia	30, August 2017	Up to 25 years of age
Breyanzi(lisocabtagene maraleucel)	Relapsed or refractory large B-cell lymphomas	5,February 2021	Adult
Tecartus (brexucabtagene autoleucel)	Relapsed or refractory mantle cell lymphoma (MCL).	24, July 2020	Adult

Table 4: Current clinical targets of CAR-T therapy for hematological malignancies (ClinicalTrials.gov).(L. Zhao & Cao, 2019)

Target	Disease	Stage	Phase	NCT number	Country
CD19	Refractory CD19+ lymphoma and leukemia	Completed	I	NCT01626495	United States

	B-cell leukemia or lymphoma		I	NCT01029366	United States
	B cell leukemia or lymphoma		I	NCT01593696	United States
	ALL		I	NCT01551043	United States
	Resistant or refractory CD19+ ALL		II	NCT02030847	United States
CD19 + CD22	Relapsed or refractory lymphoma and leukemia	Recruiting	I	NCT03233854	United States
	CD19 positive diffuse large B-cell lymphoma or B ALL				
	CD19 +CD22+ leukemia		I	NCT03330691	United States
	Children or young adults with CD19 positive B ALL		I	NCT03241940	United States
	CD19/CD22-expressing B cell malignancies		I	NCT03448393	United States
CD22	CD22+ leukemia and lymphoma	Active	I	NCT03244306	United States
	Chemotherapy resistant or refractory ALL	Terminated	I	NCT02588456	United States
	ALL	Recruiting	I	NCT03620058	United States
	ALL	Recruiting	I	NCT02650414	United States
CD4	CD4+ lymphoma and leukemia	Recruiting	I	NCT03829540	United States

Table 5: Current clinical targets of CAR-T therapy for solid tumors (ClinicalTrials.gov)

Target	Disease	Stage	Phase	NCT number	Country
Mesothelin	Malignant pleural mesothelioma	Completed	I	NCT01355965	United States
	Metastatic pancreatic (ductal) adenocarcinoma	Completed	I	NCT02159716	United States
ss	Epithelial ovarian cancer	Completed	I	NCT01897415	United States
	Recurrent or refractory pediatric CNS tumors	Recruiting	I	NCT03638167	United States
	HCC	Recruiting	I	NCT02905188	United States
HER2	Brain or leptomeningeal metastases	Recruiting	I	NCT03696030	United States
	HER2+ CNS tumors	Recruiting	I	NCT02442297	United States
	Recurrent/refractory pediatric CNS tumors	Recruiting	I	NCT03500991	United States
CEA	Metastatic pancreatic carcinoma	Recruiting	I	NCT03818165	United States
	Liver metastases	Completed	I	NCT02416466	United States

3.5 Cancer Vaccines

In the last decade, cancer vaccines have been said to have found their way in the field of oncology. These Cancer vaccines are now considered as one of the greatest achievements among other major approaches that are currently presented as immunotherapeutic treatment for cancer patients. The composition of cancer vaccines mainly involves fragments or whole of cancer cells, or antigens coupled with an adjuvant before administration which are designed to stimulate the immune response in patients. These vaccines are administered to patients through vaccination thus triggering immune response (Sambi et al., 2019). The vaccines are categorized into two major types. These include therapeutic vaccines and preventive vaccines. Therapeutic vaccines directly target and broaden the immune system's attack on cancer cells by inducing and producing autoimmune responses in tumor-affected areas, while Preventive vaccines are those that, for the most part, prevent the development of cancer. These antigen-based vaccines contain infectious agents that are easily recognized as foreign substances, stimulating the immune system with tumor antigens while activating the immune system with targeted T Cells to eliminate cancer cells. Human papillomavirus and Hepatitis B virus vaccines are examples of preventative vaccines approved by the FDA (H. Zhang & Chen, 2018).

Mechanism of Action of Cancer Vaccines

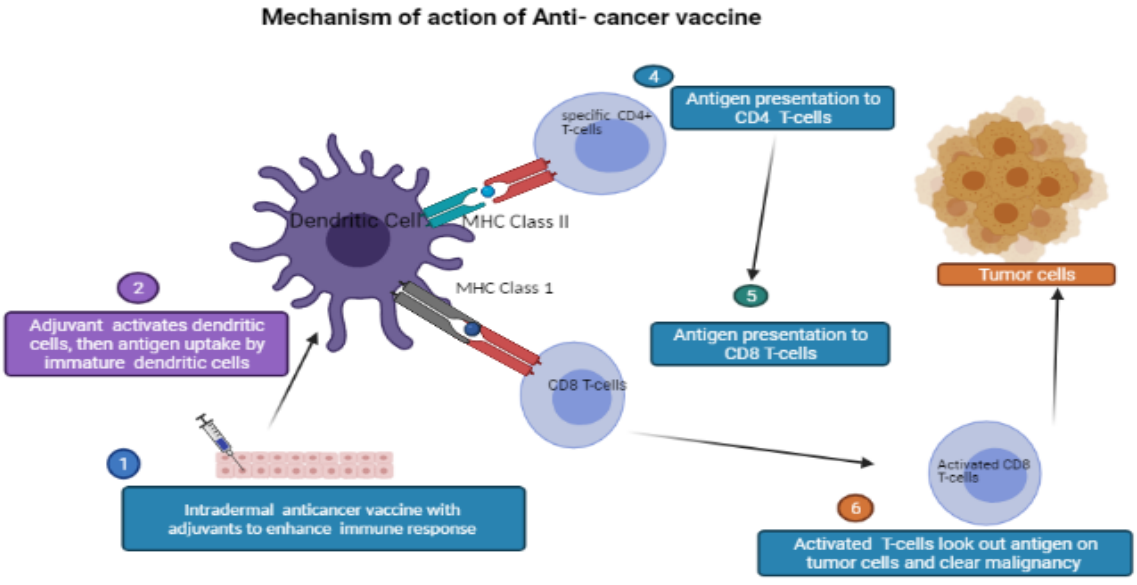

Figure 4: Mechanism of action of cancer vaccines (Adapted from (Sambi et al., 2019))

Cancer vaccines are given intradermally to cancer patients with infection, together with adjuvants that stimulate dendritic cells. Dendritic immature cells bind to an antigen commonly exhibited on tumor cells and the antigen is then and it's taken to lymph node to be presented to cluster differentiation -4 cells (CD4 cells) and CD8 cells, which are stimulated for recognizing these antigens on the surface of the tumor cells and destroy them (Sayour et al., 2018).Tumor antigens are fully presented by major histocompatibility complexes molecules found on the APC surface allowing them to be recognized by T cell receptors in order to elicit an anti-tumor immunological reactions by the naive CD8+ T-cell surface primed into Cytotoxic T-lymphocytes. As a result, the cytotoxic effects are exerted, and the tumor cells are killed (Qin et al., 2018).

3.5.1. Current Application of Cancer Vaccines

The FDA has approved some therapeutic cancer vaccines, particularly those that have shown positive clinical trial results. The recently approved therapeutic cancer vaccines that target tumor-specific antigens (TAAs) expressed in tumor cells. Among these approved therapeutic cancer vaccines is Sipuleucel-T (PROVENGE) from Dendreon Corporation, which was approved by the FDA in April 2010 as a cellular immunotherapy. Sipuleucel-T was developed for the treatment of metastatic castration-resistant prostate cancer, but it was rejected by the FDA in 2007 after two phase III trials as it failed to achieve the primary goal of progression-free survival in cancer patients. In addition, a 33 percent reduction in the risk of death for the PROVENGE cancer vaccine in cancer treatment is demonstrated by analysis from numerous clinical trials (DeMaria & Bilusic, 2019).

3.5.2. Challenges of Cancer Vaccines.

The difficulties encountered by these vaccine therapies include tumor heterogeneity and low antigenicity of the targeting antigen (Ye et al., 2018). MHC class I expression is also low on tumors, which affects tumor cells' ability to present antigens for killing by CD8 T cells (Science, 2020). The short duration of an antitumor response following vaccination may result in low vaccine response rates and a weak antitumor curative effect (Qin et al., 2018).

3.5.3. Pharmacodynamics (Potency, Efficacy and Specificity)

The efficacy of a cancer vaccine is determined by the different expression of target antigens by tumor cells (Ye et al., 2018). Cancer vaccines have shown promise in clinical trials, for example, therapeutic cancer vaccines have proven to be effective anticancer agents with low toxicity (DeMaria & Bilusic, 2019) .These cancer vaccines are antigen specific due to tumor-specific antigens expressed by cancer cells, making them excellent candidates for anti-cancer therapy (Schietinger et al., 2008).

3.5.4. Side Effects of Cancer Vaccines

Cancer vaccine side effects are said to vary from vaccine to vaccine and from patient to patient, so (inflammation) mild acute infusion reactions are the most commonly reported side effect of the vaccine at the site of infection (Thomas & Prendergast, 2016). To control such side effects, prophylactic treatment, as well as infusion rate adjustments, is advised.

Table 6: Therapeutic cancer vaccines are currently FDA-approved for the treatment of early-stage bladder cancer, mCRPC, and metastatic melanoma (Rousseau et al., 2001)

Brand Name &(Generic Name) of Cancer Vaccine Therapy	Therapeutic indication	Date of authorization (FDA)
Sipuleucel-T (PROVENGE®)	Metastatic castration resistant prostate cancer (mCRPC)	April 2010

3.6 Enzyme Immunotherapeutics

Enzymes have found their way into immunotherapeutics because of high specificity in their mode of action, this has attracted their use and recommendation as a therapeutic after several researches. Since the 1960s, some enzymes have failed to reach treatment therapy although others have shown promise, and the FDA has approved up to 15% of proteins which are enzymes to be used for therapeutic applications such as cancer treatment, digestion, and clot lysis (Nunes et al., 2020). They are now presented as successful immunotherapeutics due to their clinical successes and abilities to catalyse substances that may induce /activate or

inactivate/ suppress immune response signals. Secondly enzymes are also able to produce a sustained biocatalysis for the required time scale as they are administered after their modification. Enzymes are proteins that transform multiple copies of substrate molecules into a variety of products in a rapid and precise manner (Farhadi et al., 2018).Tumors take advantage of these metabolites to facilitate the spread of cancer cells and weaken the immune response to tumors (Henderson, 2020). It uses a deamination process as it catalyses L-asparagine to Aspartic acid and ammonia. L-Asparaginase enzyme is used as an anticancer drug because it has anticarcinogenic potential and is primarily used in the treatment of acute lymphoblastic leukemia in children and adolescents. The asparaginase presence was reported in 1922 in blood serum of a guinea pig by Clementi while asparaginase tumor inhibitory was described by Kidd in 1953 with almost total or rapid tumor regression after treating lymphoma bearing mice with guinea pig serum (Nunes et al., 2020).

3.6.1. Mechanism of Action Enzyme Therapy

Enzyme immune therapeutics in cancer therapy is a therapy that is used to degrade the substrate into products to render the tumour cells to be susceptible to immunotherapy. Of all the proteins, the L- asparaginase enzyme is the only FDA-approved enzyme therapy, and it's the only enzyme therapy that's recommended for treating acute lymphoblastic leukemia (Farhadi et al., 2018). Leukemic cells express low levels of asparaginase synthetase, they have a deficiency to produce more when exposed to asparaginase. Leukemic cells have an extreme high sensitivity to the deficiency of extracellular asparagine which causes disruption of asparagine dependent protein synthesis (Koprivnikar et al., 2017). Cancer cells especially of lymphoid origin depend on exogenous L-asparagine which is supplied from blood serum for their metabolic activities like metastasis, malignant growth and survival. Hence, asparaginase hydrolysation using L-asparaginase enzyme from the blood

serum causes cancer cell P53 dependent apoptosis which in turn inhibits cell proliferation of these tumor cells while leaving healthy cells unaffected (Nunes et al., 2020).

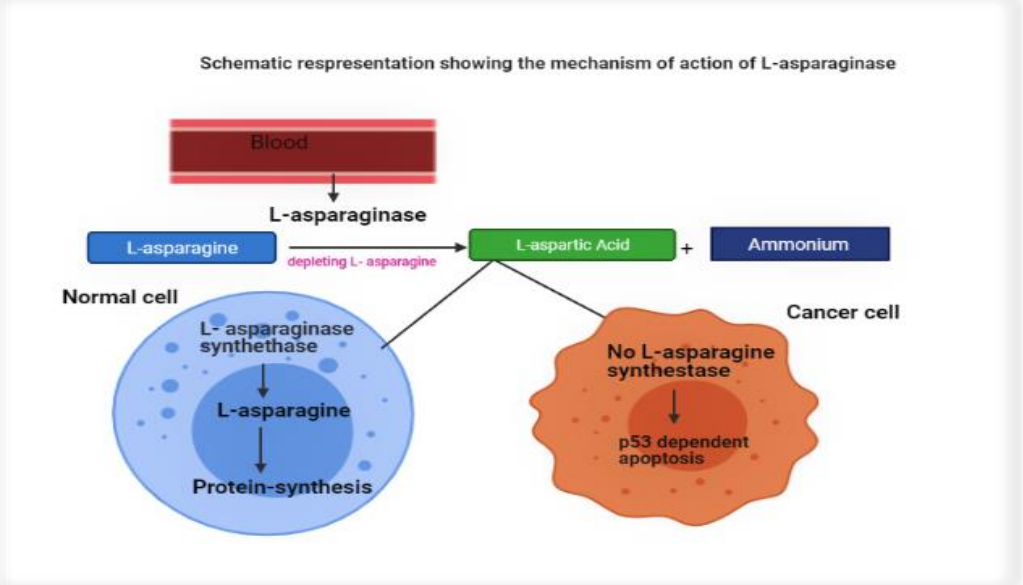

Figure 5: Mechanism of action of L-asparaginase (adapted from(Nunes et al., 2020))

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3.6.2. Current Clinical Applications in Oncology

Because of its anticarcinogenic properties, L-asparaginase, which is derived from E. coli, is widely used in the clinical treatment of lymphoproliferative disorders and acute lymphoblastic leukemia (Nunes et al., 2020). Patients are given either an intramuscular injection or an intravenous infusion of E. coli L-asparaginase, which operates at pH 4–5. The investigation of the application in adults with leukemia, means that the drug is administered intravenously or intramuscularly, with a daily dose of 25,000 U/m2 or a 6,000 U/m2 diet for 6 days. Moreover, taking 2,500–5,000 U/m2 from Day 3 to Day 8 is effective in lowering serum asparagine in infants (Ghasemian et al., 2019).

3.6.3. Pharmacodynamics (Potency, Efficacy and Specificity)

This therapy is successful and secure, particularly in the treatment of young adults with acute lymphoblastic leukemia, with a response rate of 78 percent to 96 percent in the front line, compared to other trials that show a four-year overall survival rate of 50% or better. However, data from a recent study shows promising results in the adolescent and young adult population (Koprivnikar et al., 2017). It is a highly favored therapy because of its biodegradability and non-toxicity as opposed to other therapies because it targets only certain leukemic cells that have a deficiency of asparagine synthetase (Ghasemian et al., 2019).

3.6.4. Limitations of Enzyme Immunotherapeutic Therapy

Despite its successful therapeutic application, L-asparaginase should be administered with extreme care because the therapy is still said to have more unknown pieces of information that require further study. However, the major challenges posed by this therapy include; fast plasma clearance in blood as well as a short half-life due proteolytic enzyme degradation by proteases present in the body. L-asparaginase is thermolabile and thus unstable. Finally, a high number of hypersensitivity reactions, such as pancreatitis, have been reported in 30–70% of patients following administration of L-asparaginase from E.coli (Nunes et al., 2020).

3.6.5. Solution to Challenges of Enzyme Immunotherapeutic Therapy

Alternative therapy and recombinant technologies should be used to improve challenges of L-asparaginase such as hypersensitivity, resistance, immunogenic complications, and enzyme short life through physical integration and chemical modification (Ghasemian et al., 2019). Therefore using strategies like L-asparaginase confinement in several nanomaterials using methods like entrapment, physical adsorption, and covalent attachment methods which have improved its properties like half-life enhancement, increased resistance to proteolysis,

specificity, improved thermal and operating stability, allowing re-use of these enzymes and reducing of costs (Nunes et al., 2020).Chemical modification strategies, such as PEGylation, can be used to improve the efficacy of enzymes in the treatment of cancer, such as PEGylation of L-asparaginase for the treatment of acute lymphoblastic leukemia (O'Donnell et al., 2018)."Polyethylene glycol (PEG) is recognized as a material for immune response prevention without affecting enzyme anticancer properties," said the researchers (Ghasemian et al., 2019).Lymphoblastic leukemia cells reduce their ability to synthesize L-asparagine, making them more vulnerable to chemotherapeutic-induced death in asparagine-deficient plasma. However, with infusion doses of L-asparaginase, an enzyme that degrades L-asparagine, and a half-life of 12 hours in blood by recombinant L-asparaginase, PEGylation is used to increase L-asparaginase activity a half-life to about 6 days (O'Donnell et al., 2018).

3.6.6. L-Asparaginase Toxicity/Adverse Effects

When L-asparaginase therapy is not administered with caution, it can result in a number of toxicities, including anaphylaxis and sudden death (Nunes et al., 2020) .

Table 7: Enzyme therapy for treatment of cancers approved by FDA (Farhadi et al., 2018)

Enzyme therapy for treatment of cancers approved by FDA (Farhadi et al., 2018)

Brand Name &(Generic Name) of TIL Therapy	Therapeutic indication	Date of authorization (FDA)
L- asparaginase enzyme	Acute lymphoblastic leukemia(ALL)	18, November 2011

3.7 Checkpoint Inhibitors (CTLA-4, PD-1, PD-L1)

Immune Checkpoint Inhibitors are monoclonal antibodies that inhibit immune checkpoints in order to regulate T-cell-mediated tumors (Wang et al., 2017) and they work by disabling the "breaks" of the body's natural defense against cancer and T cells. Immune checkpoints contribute significantly to the immune system by sustaining the body's immunological balance and self-tolerance in relation to immune-oncology. There are three major immune checkpoints: programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD- L1), as well as cytotoxic T lymphocyte-associated antigen (CTLA-4). Several preclinical studies have revealed that inhibiting CTLA-4 and PD-1/PD-L1 checkpoints improves and accelerates the immune system's anti-tumor properties; thus, these checkpoint blockades possess a high chance of success in cancer treatment. However, current data show that only about 20% to 30% of patients treated with ipilimumab had a higher survival rate, with toxicities taking place within a sizable patient population. In addition to that, initial findings from anti-PD-1 clinical trials therapy, Pembrolizumab, for example, have been shown to have higher objective anti-tumor response rates than ipilimumab in metastatic melanoma. As a result, pembrolizumab was approved by the FDA for the treatment of non-small cell lung cancer (NSCLC) and squamous cell carcinoma of the head and neck. Furthermore, Nivolumab, the FDA's second anti-PD-1 mAb approval, has demonstrated efficacy in the treatment of various types of cancer, including NSCLC, melanoma, and renal cell carcinoma (RCC), with patients experiencing rapid and long-lasting tumor regression (Das et al., 2017). Immune checkpoint inhibitors are currently regarded as the most effective immunotherapeutic approach due to their unique ability to target lymphocyte receptors when compared to Trastuzumab, bevacizumab, and cetuximab which are examples of targeted therapies that act directly on tumor cells (F. De Felice et al., 2018).When tumor cells express PD-1 ligands, PDL1 binds to PD1, causing T cell function to be downregulated and a

negative feedback loop develops reducing anti-tumor immunity. Therefore, inhibiting PD-1 and its ligands restores T cell effector activation function and tumor cell eradication.CTLA-4 inhibitors:These CTLA-4 receptors are found on the T cell surface e.g on the T-helper and T-killer cells and compete with the CD28 receptor for the binding of CD80 or CD86, thereby inhibiting T cell activation, also Signaling by CTLA-4 boosts T-regulatory cells' inhibitory function. Several CTLA-4 blockers have been tested in clinical trials, including blocking monoclonal antibodies such as tremelimumab and ipilimumab (YERVOY), which are humanized anti-CTLA-4 mAbs that bind to CTLA-4 and inhibit interactions with CD86/80 (B7-2)/ (B7-1).Tremelimumab, for example, is a CTLA-4 inhibitor being studied in clinical trials for a variety of cancers. The US Food and Drug Administration approved Ipilimumab in 2011. as the first immune checkpoint inhibitor in the treatment of melanoma patients, but it is now being studied in 17 clinical trials to determine its efficacy in Head and Neck Squamous Cell Carcinoma (HNSCC) (Francesca De Felice et al., 2019).These drugs' mechanism of action involves the activation of cytotoxic T cells, which can result in a Insufficiency of self-tolerance and adverse immune-related events (Johncilla et al., 2020). Programmed cell death-1 and programmed cell death Ligand-1: PD-1 is a surface receptor expressed by T cells that promotes apoptosis of antigen-specific T cells while decreasing apoptosis of regulatory T cells via interaction with its ligand. PDLI is a receptor that tumor cells develop and use to counteract the immune cell response by turning off the immune system, specifically activation of T Cells. Inhibitors of PD-1 and PD-L1 Block the PD-1–PD-L1 interaction, allowing T cells to activate and survive to cause immune response (Ramos-Casals et al., 2020).The PD-1 signaling pathway allows for the maintenance of T cell tolerance in the body (Wakeley et al., 2020).When a T cell recognizes the antigen expressed by the MHC complex on the target cell, inflammatory cytokines are released, kicking off the inflammatory response (Mahoney et al., 2015).To activate once more exhausted T Cells, and those turned off by

signals produced by its ligand from tumor cells, anti-PD-1 and anti-PDL-1 monoclonal antibodies are used. Two distinct signals are responsible for T cell activation. The first is antigen recognition by T cell receptors (TCRs) immediately following antigen presentation by MHC class II molecules. Second, CD86 costimulatory signals activate T-cells, which then migrate to tumor sites, where tumor neoantigen recognition increases PDL-1 expression on tumor cells, causing T cell exhaustion due to increased PDL-1 binding with PD-1 receptors, sending signals that inhibit T cell activation.

Mechanism of Checkpoint Inhibitors

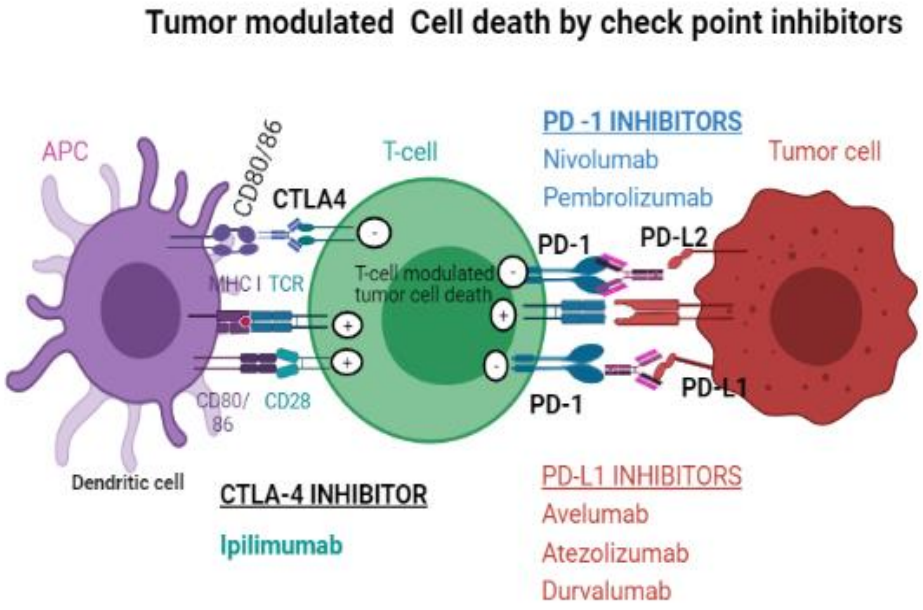

Figure 6: Mechanism of action of immune checkpoint inhibitors (adapted from (Centanni et al., 2019))

.Checkpoint inhibitors inhibit the checkpoints that regulate T cell activation, allowing them to stay in action and destroy cancer cells, thus defeating the cancer cells' mechanism for evading immune surveillance. After activation, activated T cells for the immune system present a regulatory mechanism in which the T Cells surfaces express a protein molecule checkpoint called CTLA-4, which is similar to the CD28 molecule. CD28, on the other hand, acts as a costimulatory signal for T cell activation, whereas CTLA-4(CD 152) acts as an inhibitory signal

molecule a . It is said to have a greater affinity for CD80 (B7-1) and CD86 (B7-2), and it outcompetes CD28, resulting in less pro-effector cytokine release (Vaddepally et al., 2020). Programmed Death-1(B7-H1) binds to the Programmed Death ligand-1.Programmed Death-Ligand-1(B7-H2) is present on the surface of a variety of tissue types, including several Tumors and hematopoietic cells. The PD-1and PD-L1 direct interaction results in tumor cell apoptosis inhibition, peripheral T effector cell exhaustion, and T effector cell conversion to regulatory T cells. However, using anti-PD-1 or anti-PDL-1 monoclonal antibodies prevents interaction between them, enhancing T cell anti-tumor activity by triggering an immune response to activate production of T cells to control and destroy cancerous cells (Vaddepally et al., 2020).

3.7.1. Current Applications of Checkpoint Inhibitors

When it comes to the treatment of solid tumors including melanoma, renal cell carcinoma, urothelial carcinoma, and head and neck squamous cell carcinoma, checkpoint inhibitors have had a lot of performance .For example, Ipilimumab acting as anti-CTLA-4 is used to treat advanced melanoma patients and it has demonstrated a high overall survival rate in patients who had previously been treated for metastatic melanoma. Anti-PDL1 antibodies, such as Avelumab, Durvalumab, and Atezolizumab, are used to inhibit the interaction between the two receptors. Anti-PD-1 monoclonal antibodies Pembrolizumab and Nivolumab have been recommended for the treatment of advanced melanoma in 2014.Since PD-L1 is expressed on 50% or more on cancer cells, pembrolizumab is the first-line treatment for NSCLC with high PDL1 expression .Renal cell carcinoma patients who have advanced disease have been noted to be having a positive response with nivolumab whose report in phase 3 showed overall survival (Ottaviano et al., 2019).

3.7.2. Challenges of Checkpoint Inhibitors

Identifying the right patient to treat with immunotherapy remains a significant challenge of this therapy. However, the presence of biomarkers are capable of predicting a response to a given regimen (Lleo et al., 2019). On the other hand, failure of the patients to positively respond to checkpoint inhibitor treatments because of resistance intrinsically or disease progression after the initial response is another challenge. Lastly, the mechanism of treatment is still unknown (Ottaviano et al., 2019).

3.7.3. Side Effects of Checkpoint Inhibitors

These include kidney infections, itching and rashes, diarrhea, hormonal liver problems, and lung inflammation (Pneumonitis) and the side effects can be avoided if the doctor delays administering checkpoint treatments to a patient for a set amount of time for the body to recover; additionally, minor side effects can be mitigated with medications (American Cancer Society, 2020).

Table 8:Approved Immune Checkpoint Inhibitors (Centanni et al., 2019)

Generic name (receptor target)	Therapeutic indication	Date of authorization (FDA)
Ipilimumab (CTLA4)	Melanoma	March 2011
	Renal cell carcinoma Colorectal cancer	April 2018 November 2018
Atezolizumab (PDL1)	Urothelial carcinoma Nonsmall cell lung Cancer	May 2016 October 2016
Avelumab (PDL1)	Merkel cell carcinoma Urothelial carcinoma	March 2017
Durvalumab (PDL1)	Urothelial carcinoma Nonsmall cell lung Cancer	May 2017 February 2018
Nivolumab (PD-1)	Melanoma	December 2014
	Nonsmall cell lung Cancer	October 2015

	Renal cell carcinoma	November 2015
	Classic Hodgkin lymphoma	May 2016
	Squamous cell cancer of the head and neck	November 2016
	Urothelial carcinoma	February 2017
	cancer Colorectal Cancer Hepatocellular carcinoma	September 2017
Pembrolizumab (PD-1)	Melanoma	September 2014/ July 2015

Table 9: Checkpoint Blockade Targets in Clinical Trials (H. Zhang & Chen, 2018)

Target	Drug name	Cancer types	Current Status
CTLA-4	Ipilimumab	Multiple cancers	Phase I-III
	Tremelimumab	Multiple cancers	Phase I-III
PD-1	Nivolumab	Multiple cancers	Phase I-III
	Pembrolizumab	Multiple cancers	Phase I-III
	MED10680	Multiple cancers	Phase I
	AMP-224	Multiple cancers	Phase I
	Pidilizumab	Multiple cancers	Phase I-II
PD-L1	Atezolizumab	Multiple cancers	Phase I-III
	MED14736	Multiple cancers	Phase III
	Avelumab	Multiple cancers	Phase I-III
	BMS-936559	Multiple cancers	Phase I

3.8 Monoclonal Antibodies

Monoclonal antibody (mAb) was invented in 1975 by Kohler and Milstein together with development of Hybridoma technology which revolutionized monoclonal antibody therapy; this led to production of specific antibodies that targets tumor neoantigens.Approximately ten (10) monoclonal antibodies in 1997 got approved by the US Food and Drug Administration (US-FDA) to treat a wide range of solid tumors and haematological malignancies with many

other therapeutic antibodies in clinical trials (Pillay et al., 2011). Antibodies are substances which are naturally produced by the body for defense as they recognize foreign substances such as germs and tumors and mark them for destruction. Monoclonal antibodies (mAbs) being monospecific, are used effectively in disease diagnosis and therapy. Monoclonal antibodies are proteins of the immune system which are manufactured in the laboratory from a single clone of a cell and are used to recognize and interact with specific targets according to the National Cancer Institute (NIH). Monoclonal antibodies immune-therapeutically perform a great function in cancer treatment. For example, enhancing the immune system's response to cancer by labeling cancer cells for better recognition e.g Rituximab, binds to a protein called Cluster of Differentiation (CD20) on B cells and some cancer cells, causing the immune system to destroy them. Monoclonal antibodies bring T cells close to cancer cells, allowing the immune cells to kill them. For example, Blinatumomab (Blincyto®) binds to both CD19, a protein found on the surface of leukemia cells, and CD3, a protein found on the surface of T cells that allows T cells to get close enough to the leukemia cells to destroy and kill them.Trastuzumab, a humanized epidermal growth factor receptor 2 (HER2) mAb for the treatment of HER2-positive breast cancer, which accounts for 25% to 30% of all breast cancers, was the US Food and Drug Administration's first successful monoclonal anticancer antibody. In the treatment of breast cancer, they can be used in combination, for example, the first homo-combination of two mAbs, each engaging a distinct site of HER2, and a hetero-combination of antibodies to two distinct T-cell antigens (Peterson et al., 2018). Several antibodies targeting checkpoints are being developed to boost T cell activation and kill cancer cells. It has been shown to be especially effective against neoantigens (Van den Bulk et al., 2018).Monoclonal antibodies are used in cancer treatment and have a significant impact as it directly or indirectly induces the tumor cell destruction by targeting the tumor or the vasculature that nourishes the tumor. Cancer cells have specific targets which are specifically

targeted by these antibodies, for example, epidermal growth factor receptors (EGFR), carcinoembryonic antigen (CEA) related to colorectal and, lung; breast cancer related to HER2/neu, and CD20 on cells of B that are a marking for non-Hodgkin's lymphoma (NHL). Approved therapeutic antibodies to ERBB2 and CD20 include rituximab (Rituxan; IDEC pharmaceuticals) for NHL. When used in conjunction with conventional chemotherapy therapies, monoclonal antibody therapy is said to be successful thus Ibritumomab tiuxetan (Zevalin; IDEC) and Gemtuzumab ozogamicin, two new chemotherapy antibodies, have been approved by the FDA for use in treatment of cancer (Mylotarg; Wyeth).

Schematic diagram showing monoclonal Antibody production

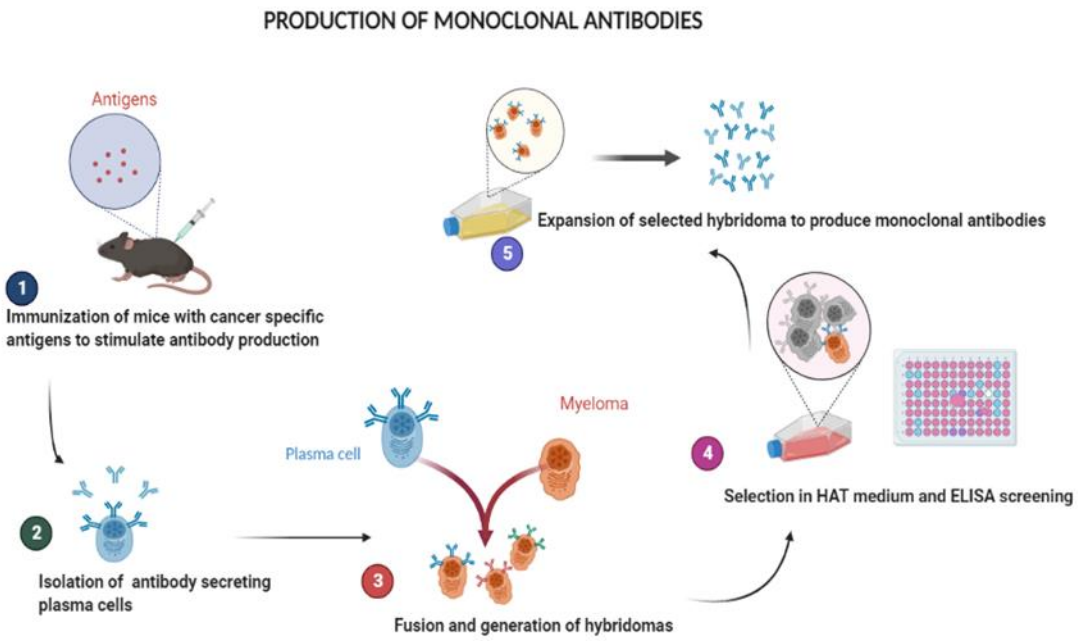

Figure 7: Production of monoclonal antibodies (adapted from (Brekke & Sandlie, 2003))

3.8.1. Types of Antibodies used in Cancer Treatment

3.8.1.1 BispecificMonoclonal Antibodies

Blinatumomab (Blincyto) is a drug that is used in the treatment of some types of leukemia. These drugs contain two different mAb that at the same time, attach to two distinct proteins,a part binds to CD19 protein, which is expressed on leukemia and lymphoma cells and the other component binds to CD3, a protein found on immune T cells .e.g, Blinatumomab combines cancer cells and immune cells, by binding to both of these proteins, the immune system is activated, causing an immune response to attack cancer cells (Gasser & Waaga-Gasser, 2016).

3.8.1.2 Naked Monoclonal Antibodies

They are the most conventional natural mAbs used to treat cancer because they can recognize and bind to cancer cell neoantigens.These antibodies do not contain any drugs or radioactive substances; instead, they strengthen a person's immune system by linking to cancer cells and serve as an indicator for the body's immune system to eliminate them. For instance, alemtuzumab (Campath®), a medications used to treat chronic lymphocytic leukemia in some patients .Alemtuzumab binds to the CD52 antigen present on lymphocytes, which includes leukemia cells.When the antibody binds to the target cell, immune cells attack it (Gasser & Waaga-Gasser, 2016).

3.8.1.3 Conjugated Monoclonal Antibodies

These types of antibodies are subdivided into two, Radio labeled (radio immunotherapy) antibodies and Chemo Labeled antibodies. Conjugated mAbs are those that are in conjunction with a chemotherapeutic agent alternatively, it may be a radioactive particle. Conjugated monoclonal antibodies serve as a vehicle for delivering these substances to cancer cells

directly. They travel around the body until they find and bind to the antigen of interest. It also transports hazardous substances, such as cytotoxic drugs, to the required location .As a result, regular normal cells in other parts of the body are less damaged. A radiolabeled monoclonal antibody example is Ibritumomab tiuxetan (Zevalin).This antibody is directed against the CD20 antigen, which is present on B cells lymphocytes. These antibodies directly deliver radioactive substances to sites with cancerous cells. It contains both a monoclonal antibody drug (Rituximab) and a radioactive element (Yttrium-90). Chemo labeled antibodies include; the antibody Brentuximab vedotin (Adcetris) that targets the lymphocyte antigen CD30 and is linked to the chemotherapy drug Monomethyl Auristatin ado-trastuzumab emtansine (MMAE) (Kadcyla, also called TDM- 1).These monoclonal antibody conjugated therapies are said to have greater efficacy in cancer treatment while having a significant reduction in toxicities (Coulson et al., 2014).

3.8.2 Forms of Monoclonal Antibodies

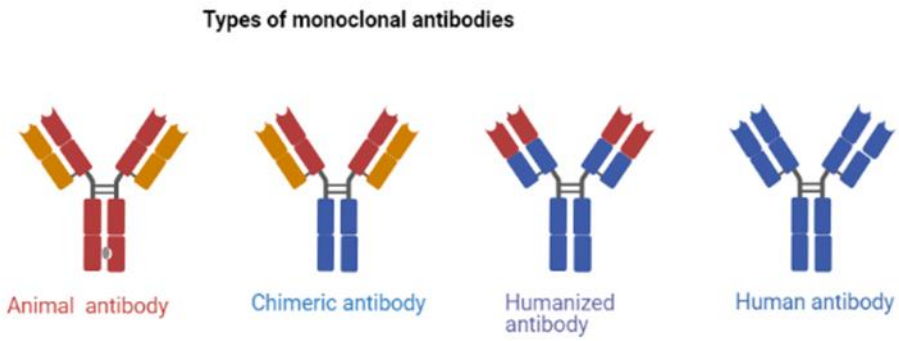

Figure 8: Types of monoclonal antibodies (adapted from (Jarboe et al., 2014))

The chimeric monoclonal antibodies are antibodies that have variable murine origin regions and constant human origin regions. Human monoclonal antibodies are monoclonal antibodies that are obtained entirely from body cells. Humanized monoclonal antibodies are antibodies except for the portion of the antibody that recognizes and binds to its target (binding site),It

comes from sources that are human.e.g, The antitumor agent calicheamicin is linked to gemtuzumab ozogamicin, a monoclonal antibody that has been humanized. It has been approved for use in patients with recurrent Acute Myelogenic Leukaemia, and it is directed against CD33, which is expressed in approximately 90percent of all Acute Myelogenic Leukaemia cases.Murine monoclonal antibodies are derived entirely from a murine source (Lym-1; Peregrine Pharmaceuticals). Ibritumomab tiuxetan antibody is a radioisotope 90 Yttrium attached anti-CD20 mouse antibody that targets B cell tumors on mature B cell surfaces, causing cellular damage. It's the very first FDA-approved radioimmunotherapy antibody medication for non-Hodgkin lymphoma treatment. Hybridoma technology, which includes the fusion of immortalized myeloma cells with immunized mouse B cells, is used to generate antibodies in mice.The antibody-producing murine cells are isolated and fused with myeloma cells and laboratory grown to form hybrid cells called hybridomas (Pillay et al., 2011).

3.8.3. Mechanism of Action of Antibodies

Patients administered with chemotherapeutic monoclonal antibodies are able to achieve their therapeutic effects through when these antibodies attach to the specific target antigen tumor and trigger cell destruction through using three major mechanisms. Firstly, they can cause direct tumor death death by blocking cell survival signalling the induction of apoptosis by body’s immune system e.g in conjugated antibodies can directly deliver radioisotopes or cytotoxic drugs to the tumor site .Secondly, vascular disruption and ablation which deliver nutrients and spread of cancer for their growth and development this inhibits the cancer cells from getting sufficient blood supply and supporting network for tumor regression. Finally, antibody dependent cellular mediated cytotoxicity, complement mediated cytotoxicity. Also monoclonal antibodies can activate T lymphocyte cells through inhibition of T-Lymphocyte in-hibitory receptors. Chemotherapeutic monoclonal antibodies target surface antigens on the

tumor cells like antigens associated with differentiation and growth such as cluster of differentiation (CD) like CD20, CD 30, CD 33 and CD52, carcino-embryonic antigen (CEA) (Coulson et al., 2014). For example, Trastuzumab attaches to the human epidermal growth factor receptor 2 (HER2) molecule expressed on the surface of some cancer cells, preventing HER2 from sending signals that promote cancer growth. Bevacizumab, a monoclonal antibody, inhibits VEGF, preventing the tumor from growing new blood vessels that it requires to survive. The cytokine vascular endothelial growth factor (VEGF) is a powerful angiogenesis inducer (the formation of blood vessels). Inhibitory agents, such as unique antibodies, have been developed to delay tumor growth by inhibiting VEGF induced angiogenesis. One such antibody is Bevacizumab, a humanized anti-VEGF antibody currently in Phase III clinical development for treatment breast and colorectal cancer (Das et al., 2017).

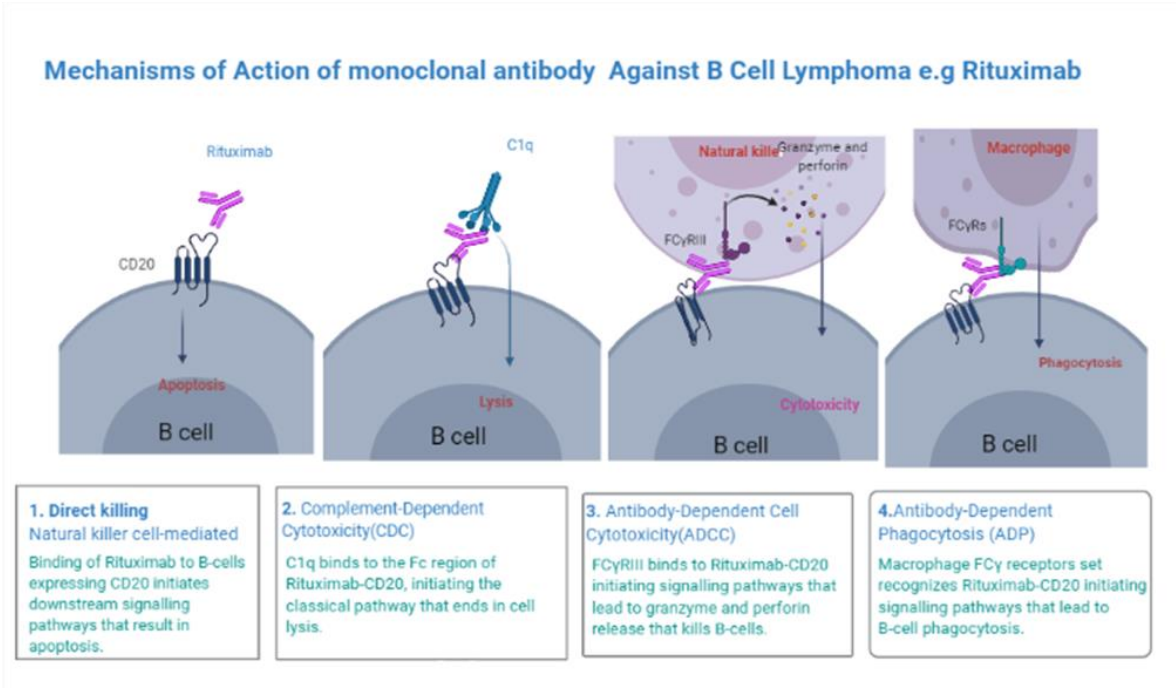

Figure 9: Mechanism of action of monoclonal antibodies (adapted from (Brekke & Sandlie, 2003))

These monoclonal antibodies work by destroying tumor cells after they have attached to the particular target antigen tumor. Their modes of action include: 1) direct tumor killing; 2)

complement mediated cytotoxicity, 3) antibody-dependent cell mediated cytotoxicity and 4) activating cellular phagocytosis through antibody dependent phagocytosis as part of tumor cell death mediated by the immune system. Immunostimulatory monoclonal antibodies can also stimulate T lymphocyte activation by inhibiting T lymphocyte inhibitory receptors. Direct involvement of tumor cell death via mechanisms such as apoptosis induction or use of conjugated antibodies for direct distribution of cytotoxic drugs or radioisotope modalities to the target tumor neoantigen and inhibition of cell survival signaling, vascular ablation and disruption of connective tissue connections with cancer cells cut off the tumor's blood supply, allowing the tumor to shrink (Coulson et al., 2014). Pembolizumab is a humanized monoclonal antibody that blocks immune checkpoints on immune cells, allowing immune cells to become stimulated and triggered to eliminate cancer and destroy it. Finally monoclonal antibody treats cancer by acting as markers cancer cells for their destruction, for example, Rituximab attaches to CD20 on the cancer as it is able to act as a marker on the cancer cells which the immune system recognizes and destroys it (Mylotarg; Wyeth).

3.8.4. Current Applications of Antibody Therapy

Monoclonal antibodies are used in overcoming cancer resistance and are used in blocking the signaling mechanisms for immune checkpoints such as programmed cell death-1 and cytotoxic T lymphocyte antigen-4 and it has demonstrated clinical efficacy in a haematological malignancies, and solid malignancies (Hu et al., 2018). Monoclonal antibodies serve as delivery vehicles for harmful substances, such as cytotoxic drugs, to the appropriate site because they directly bind to the antigen of interest, minimizing damage to healthy tissue (Coulson et al., 2014).

3.8.5. Pharmacodynamics (Potency, Efficacy and Specificity)

Due to their specificity, monoclonal antibodies have shown great effectiveness as cancer therapeutics by using antibody-based cancer treatment that focuses on tumor surface antigens. However, researchers are also looking at how to target immune cells without concern for tumor antigens in order to enhance antitumor immune responses (Weiner et al., 2012).

3.8.6. Limitation/ Challenges of Monoclonal Antibody Therapy

Monoclonal antibody therapy in human's treatment of cancer, has several challenges including loss of monoclonal antibody potency, tumor antigen heterogeneity, loss of antibody tumor specificity, antigen modulation, and unclear pharmacology (Scheinberg, 1991). There are not enough biomarkers to figure out which patients might profit from monoclonal antibody therapy. Resistance is also developed to these therapy as they may contain cytotoxic drugs on them as repeated doses are given during treatment of antibody based drugs and lastly the heterogeneous nature of human malignancies may also lead to a short duration response for antibody-based drugs in patients (Modjtahedi et al., 2012). Furthermore, the cost of producing Monoclonal antibodies is extremely costly. In vitro, monoclonal antibodies can have a variety of modes of action, and when administered into patients, the exact mode of action is not always clear (Chames et al., 2009). Most monoclonal antibodies are given in big doses, ranging from 5 to 20 milligrams per kilogram body weight at a time, to attain a therapeutic plasma concentration of several hundred milligrams per milliliter over an extended period of time. As a consequence, it is possible that this could cause toxicity in patients (Samaranayake et al., 2009).

3.8.7. Solutions to Challenges of Monoclonal Antibody Therapy

Gene transfer technology is being used to create in vivo therapeutic antibodies with the aim of genetically delivering genes for clinical monoclonal antibodies to patients in order to avoid

the expensive and time-consuming intravenous mAb infusions that are currently used in this process (Samaranayake et al., 2009).

3.8.8. Monoclonal Antibody Therapy Side Effects.

Intravenous administration of monoclonal antibodies as proteins can induce allergic reactions, including chills, fever, vomiting, nausea, low blood pressure, diarrhea, and rashes. The risks of using monoclonal antibodies are severe. Cetuximab (Erbitux) medication, for example, may cause severe rashes in patients because it goes after a cell protein called EGFR, which is present on both normal skin cells and cancer cells. By targeting VEGF, which influences tumor blood vessel growth, Bevacizumab (Avastin) causes poor wound healing, blood clots, high blood pressure, bleeding, and kidney damage. Infusion-related reactions, such as cytokine release syndrome, can cause toxicity in patients receiving Rituximab (Coulson et al., 2014).

Table 10: FDA Approved Monoclonal antibodies for treatment of solid tumors

Monoclonal antibodies	Brand Name	Type of monoclonal antibody	Target	FDA approved
Trastuzumab	Herceptin®	Humanized	HER2	HER2-positive metastatic/non-metastatic breast cancer HER2-positive metastatic gastric or gastroesophageal junction adenocarcinoma
Pertuzumab	Perjeta®	Humanized	HER2	HER2-positive metastatic breast cancer HER2-positive, locally advanced, inflammatory, or early stage breast cancer
Cetuximab	Erbitux®	Chimeric	EGFR	Metastatic CRC HNSCC
Panitumumab	Vectibix®	Human	EGFR	Metastatic CRC
Necitumumab	Portrazza™	Human	EGFR	Metastatic squamous NSCLC
Dinutuximab	Unituxin™	Chimeric	GD2	Pediatric high risk neuroblastoma

Bevacizumab	Avastin®	Humanized	VEGF-A	Metastatic CRC Recurrent or metastatic non-squamous NSCLC Metastatic renal cell carcinoma Persistent, recurrent or metastatic cervical cancer Glioblastoma Recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer
Ramucirumab	Ciramza®	Human	VEGFR-2	Advanced or metastatic gastric or gastroesophageal junction adenocarcinoma Metastatic NSCLC Metastatic CRC
Ipilimumab	Yervoy®	Human	CTLA-4	Unresectable or metastatic melanoma Cutaneous melanoma
Nivolumab	Opdivo®	Human	PD-1	Unresectable or metastatic melanoma Metastatic squamous NSCLC Metastatic NSCLC Advanced RCC Recurrent or metastatic HNSCC
Pembrolizumab	Keytruda®	Humanized	PD-1	Unresectable or metastatic melanoma Metastatic NSCLC Recurrent or metastatic HNSCC
Atezolizumab	Tecentriq™	Humanized	PD-L1	Locally advanced or metastatic urothelial carcinoma Metastatic NSCLC
Adotrastuzumab	Kadcyla®	Humanized	HER2	HER2-positive metastatic breast cancer

3.9 Cytokines Therapy

Cytokines are proteins that are soluble and promote cell-to-cell communication in order to produce a response to a target antigen that is well-coordinated and specific. Therefore, scientists have attempted with great efforts to explore and characterize cytokines by investigating the mechanism of their signalling pathway in cancer treatment. They activate immune effector cells at the tumor site, causing cytotoxic T cells to recognize the tumor.

Several cytokines, including GM-CSF, IL-7, IL-12, IL-15, IL-18, and IL-21, are currently being tested in clinical trials for the treatment of advanced cancer. Adoptive cell therapy, which uses cytokines to create an in vitro, highly controlled environment for anti-tumor T cell expansion and proliferation, has made significant progress (Lee & Margolin, 2011). Cytokines are important regulators of the growth and activity of other immune systems and blood cells as they tell the immune system how to do its job. Cytokines have an impact on the growth of all blood cells as well as other cells that aid in the body's immune and inflammatory responses. They increase anti-cancer activity by sending signals that cause abnormal cells to die while allowing normal cells to live longer. Chemokine is one of the most common types of cytokines. A chemokine directs immune cells to a specific target. In addition, several types of chemokines, such as interleukins, interferons, tumor necrosis factors, and growth factors, are present, which are artificially manufactured in a laboratory and used to treat cancer. Their mode of delivery and administration is directly into a muscle, a vein or under the skin. Interleukins and interferons are two of the most commonly used cytokines in cancer treatment. Cytokines that have shown potential applications include interleukin-2 (IL-2) granulocyte macrophage colony-stimulating factor, interleukin-15, and interferon-alpha. IL-15 is a known mediator of long-term antitumor immunity because it promotes memory CD8+ T-cell survival, and they are capable of stimulating early T-cell proliferation and NK cell (Carlson et al., 2020).

Interleukins: Interleukins are cytokines that function as chemical messengers between white blood cells. Interleukin-2 (IL-2) promotes the growth and proliferation of immune cells. A synthetic version of IL-2 has been approved for the treatment of advanced kidney cancer and metastatic melanoma. For these cancers, IL-2 can be used alone or in combination with chemotherapy or other cytokines such as interferon-alfa (American Cancer Society, 2020). IL-2 is one of several related T cell growth factors, where during their signaling system they

result in activation and proliferation of CD8+ T Cells, CD4+ T Cells .The CD4+ Cell, more specifically the helper T Cell, is the cellular source of IL-2. The main physiological function of IL-2 is to promote the activation and proliferation of T cells and natural killer cells (NK) in a paracrine and autocrine mode because they can express the immediate affinity IL-2; thus, exposing NK cells to IL-2 results in proliferation, enhanced cytolytic activity, and the secretion of other cytokines. Furthermore, B cells express intermediate affinity IL-2 receptors and secrete IL-2 in conjunction with other cytokines, resulting in B cell proliferation and differentiation (Lee & Margolin, 2011).Interferons are proteins that aid in the body's battle against viral infections and cancer. IFN-alpha, IFN-beta, and IFN-gamma are the three types of interferon (IFN), with only IFN-alpha being used in cancer treatment by increasing certain immune cells' ability to destroy cancer cells. Second, it prevents tumors from receiving the nutrients and blood they need to develop by exerting anti-angiogenic activity (American Cancer Society, 2020), increases the MHC class I expression, also activates T and and B Cells, mediates dendritic cell maturation, and induces apoptosis (Carlson et al., 2020). In animal models for a number of cancers, some cytokines have demonstrated potent anti-tumor activity. Two cytokines, IL-2 and IFN-alpha, have shown some clinical benefit and have thus been approved by the FDA for the treatment of various cancers. In this case, IL-2 has been approved for the treatment of metastatic melanoma and advanced renal cell carcinoma (RCC) whereas Hairy cell leukemia, follicular non-Hodgkin lymphoma, and melanoma are among the cancers for which IFN-alpha has been approved to treat. The anti-tumor properties of cytokines have captured a wide range of imagination with their efficacy in the treatment of malignancies, leading to a rapid increase in the number of clinical trials that have investigated the safety and efficacy of cytokine-based drugs. This includes clinical trials involving both drug combinations with multiple other immunomodulators and single agents. However, due to the low response rate and high toxicity associated with high-dose IL-2 and IFN-

administration, the clinical use of these cytokines has shifted in favor of targeted therapy and immune checkpoint inhibitors (Berraondo et al., 2019). In the case of HNSCC, cytokines have been investigated in a number of research studies and clinical trials. When individual patients were treated with the combination of IFN-alpha, isotretinoin, and vitamin E (NCT00054561), the 5-year progression-free survival (PFS) and overall survival (OS) rates were 80 percent and 81.3 percent, respectively, as IFN-alpha increased the expression of MHC II on the macrophage, an antigen-presenting cell. In advanced HNSCC, the combination of IFN-alpha and recombinant IL-2 was also associated with an 18% response rate. Recombinant IL-2 improved disease-free survival and overall survival in HNSCC patients (Kareemaghay & Tavassoli, 2019) where these cytotoxic CD8+ T-cells expanded more rapidly and exhibited a stronger anti-tumor activity than other reported immune effector cells (Schmeel et al., 2014). Current research has shown that cytokines such as interleukins play a role in many tumor-driven molecular mechanisms, which has led to the development of several cytokine-based cancer therapies. Furthermore, advancements in cancer cell immunotherapy rely on the use of cytokines to create an in vitro highly controlled environment for anti-tumor T cell development (Anestakis et al., 2015).

3.9.1. Current Application of Cytokines in Oncology

Cytokines are essential protein molecules that play a big part in enhancing inhibition of the immune system acting as immunomodulators .The cytokines like IFN-Alpha and Interleukin-2 are currently used in cancer treatment in the following ways, IFN-alpha is used as adjuvant therapy to treat patients who are in stage II and Stage III melanoma cancer risk according to multi-institutional clinical trials which in their first stages demonstrated that improved overall survival and relapse-free survival. However, an analysis study involving 14 randomised clinical trials with 8,122 patients aged 18 and up found that IFN-alpha in 10 out of 17 comparisons, was connected to a significant increase in disease-free survival. Whereas,

4 of 14 comparisons, IFN-alpha was associated with an improvement in overall survival, implying that in Melanoma patients at high risk, IFN-alpha should continue to be the standard adjuvant treatment therapy. Interleukin-2 is said to play important roles in adoptive cell transfer therapy by enhancing and expanding T cells. Second, cell carcinoma and metastatic melanoma patients are treated with IL-2 who had previously been treated with cytotoxic chemotherapy but were no longer responsive. When high-dose IL-2 is used, studies have shown that in 15 percent to 20 percent of patients with advanced melanoma, it produces objective clinical responses as well as durable complete responses in 5percent to 7percent of these patients. With the overall durable clinical response, with a median response time of about 24 months and a long-term survival rate of more than 80 percent (Lee & Margolin, 2011).

3.9.2. Challenges of Cytokines

One of the primary challenges of cytokine therapy is degree of pleiotropism present in cytokine where they have the capability to act on a wide range of cell types to mediate a variety of effects, and sometimes contradictory, effects e.g IL-2's dual function as a powerful activator of both T effector cells and T helper cells and T regulatory cells, it is particularly well suited to interleukin-2 therapy. Furthermore, because cytokines have the same functional effects, therapeutic modification of cytokines is difficult because changes to one cytokine effect can be replaced by changes to others (Lee & Margolin, 2011).

3.9.3. Side Effects of Cytokines

Cytokine for instance, IL-2may possess side effects like flu-like symptoms which may include fatigue, chills, fever, and confusion. Patients may also experience nausea, diarrhea and vomiting; several patients on this treatment develop low blood pressure. In most cases it

is treated with other medications which are caused as a result of cytokine release syndrome (Lee & Margolin, 2011).

3.9.4. Solutions to the Side Effects

The following steps should be taken to reduce the side effects of cytokine therapy like modification of treatment with high doses of IL-2, for example, alterations in dose, route, and schedule, as well as chemical alterations of IL-2 molecular structure, which also alters its cellular targets to improve specificity (Lee & Margolin, 2011).

3.9.5. Pharmacodynamic (Potency, Efficacy and Specificity)

Cytokines play such an important role in antitumor specific immunity that they strike a balance between tumor rejection and antigen-specific effector cell activation (Conlon et al., 2019). In tumor surveillance cytokines have showed a great effect especially in a higher frequency of spontaneous tumor where they have exhibited a degree of pleiotropism as cytokines are able to exert its effects on several different types of cells in coordinating a diverse and opposing effect in there signalling (Lee & Margolin, 2011). Toxicity produced depends on the dose and associated with IFN- alpha use constitutional symptoms for example fever, fatigue, headache, symptoms of the digestive tract (Conlon et al., 2019).

Table 11: FDA Approved Treatments of Cancer (Lee & Margolin, 2011)

Brand Name &(Generic Name) of TIL Therapy	Therapeutic indication	Date of authorization (FDA)
High dose, bolus IL-2	Metastatic melanoma (MM)and Metastatic renal cell carcinoma(MRCC)	1998
IFN-Alpha	Hair Cell Leukemia(HCL)	1986

	Adjuvant therapy of Stage III melanoma	

Chapter 4

Future Prospects of Immunotherapy in Cancer

Cancer immunotherapy is one of the most exciting field that in a big way has attracted the attention of many researchers which has led to a wide exploration of this area in this new era of cancer, with expectations that they may come up with innovative approaches of manipulating the immune system to produce the most effective therapy to cancer patient because of the advantages it has over previous therapies e.g its fewer side effects and specificity in comparison to other modalities of cancer therapy (Surendran et al., 2018). As a result, these fantastic new approaches to cancer treatment are expected to be extremely successful and effective in the future. The approaches include alterations to current treatment agents, synthesis of new delivery methods for the treatment and use combinatorial therapies. Currently it is suggested that using immunotherapy with other therapy combinations could be a potent way to eradicate cancer and also a great way to produce more effective antitumor effects to those resistant cancers. Clinical data demonstrates that an overall, combination therapy could produce a complete antitumor effect with a lower risk of tumor recurrence with no metastatic progression, as well as low side effects and good outcomes hence combination therapy is projected as a big milestone that will revolutionize the future medication of cancer with enhanced efficacy compared to when therapy is administered singly for example immune point inhibitors like PD-1 and CTLA-4 (Surendran et al., 2018).Besides, combining conventional cancer therapy of immunotherapy together with targeted therapy is effectively explored with results demonstrating that a tremendous perfect synergistic effect could be produced leading to complete treatment of cancer patient e.g. Nivolumab and Ipilimumab combination reported in a number of clinical trials to be of great success (Sadozai et al., 2017). However the main challenges for this combination therapy is identifying the best suitable dosing and most efficacious combination biomarker-driven clinical trials. In the

future generation , due to tumor heterogeneity which may be both intra and inter, it is suggested that developing and analyzing the genomics of circulating cancer cells and free DNA should be established so that different cancers and their targets can be easily tracked for better treatment. Development of more effective delivery methods like use of nanoparticle constructs have proven to be effective vehicles for overcoming pharmacokinetic limitations. Some of the therapeutic modalities are seen to be easily cleared from the body without reaching the target point .As a result, the US Food and Drug Administration (FDA) has approved several nanosystems for clinical use, and many others are being investigated for clinical trials. These nanosystems modification with more effective technology is needed so that therapeutics can be targeted to tumor cells, improving cellular uptake and reducing off-target systemic toxicity. Finally, nanoparticles are said to be capable of carrying one or more therapeutic modalities, so these system delivery approaches are being investigated and they have so far presented positive results (Liu et al., 2019).The need for establishment of next-generation sequencing technologies like new bioinformatics approaches, is yet another focus many researchers are progressing in creating due to cancer being characterized as genomically unstable, its heterogeneity being the characteristics of the cancer. Next-generation sequencing (NGS) can provide novel insights into the molecular machinery inside cancer cells, allowing for the easy identification of single nucleotide variants, deletion and insertion mutations that may exist. The development of Next-generation sequencing with advances in bioinformatics algorithms that predict immunogenicity of mutated genes will lead to the development of safer, more efficient, and effective personalized cancer therapy (H. Zhang & Chen, 2018).

Chapter 5

Conclusion

In conclusion, cancer-immunotherapy, with several major approaches used in cancer treatment examples including, adoptive transfer cell therapy, checkpoint inhibitors, monoclonal antibodies, therapeutic cancer vaccines, cytokines therapy, and enzyme therapeutics, their tremendous potential, mechanisms of action, challenges, and side effects has been discussed in this review article. Finally, the future of cancer immunotherapy by elucidating on combination therapy, such as the use of combined checkpoint inhibitors in cancer treatment was also explored.

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