

# Current Practice and Approaches of Immunotherapy in Cancer Treatment

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of  
B.Sc. in Biotechnology

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## **Declaration**

It is hereby declared that

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

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## Approval

The thesis/project titled “Current Practice and Approaches of Immunotherapy in Cancer Treatment” submitted by

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## **Ethics Statement**

## **Abstract**

Cancer has been and continues to be one of the leading causes of human deaths. Thousands lose their lives to the different types of cancer each year. Cancer remains one of those invincible barriers in the health sector that humans are quite far from overcoming. As cancer remains to be incurable, works are being done every day globally to come up with better ways to treat it. Conventional methods of treatment like chemo and radio therapies have adverse effects on health and thus, new approaches are being used for cancer treatment. One such approach is the use of our body's own defense mechanism to attack and kill malignant cells. This review will highlight the different components of human immune system that are being used to treat cancer. It will give an idea of the mechanism of action of each of these components and indicate why they are better choices compared to traditional treatments. Side effects and/or challenges of each immunotherapy have also been included.

**Keywords:** Immunotherapy; Cancer; Immune system; Mechanism; Cancer treatment

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## List of Acronyms

mAb	Monoclonal Antibody
HAT	Hypoxanthine-Aminopterin-Thymidine
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
EGFR	Epidermal Growth Factor Receptor
CDC	Complement Dependent Cytotoxicity
VEGF	Vascular Endothelial Growth Factor
TLS	Tumor Lysis Syndrome
CAR	Chimeric Antigen Receptor
DC	Dendritic Cell
NK	Natural Killer
IL	Interleukin
IFN	Interferon
ROS	Reactive Oxygen Species
MMP	Matrix metalloproteinase
HMA	Hypomethylating agent
MHC	Major Histocompatibility Complex
PAMP	Pathogen Associated Molecular Pattern
DAMP	Danger Associated Molecular Pattern
APC	Antigen Presenting Cell
GM-CSF	Granulocyte-macrophage colony stimulating factor
TSA	Tumor Specific Antigen
TAA	Tumor Associated Antigen

HER2 Human Epidermal Growth Factor Receptor 2

CTA Cancer Testis Antigen



# Chapter 1

## Introduction

Cancer is one of those diseases that remains to be a daunting challenge to humankind. The different forms and types of it either claim or disrupt lives. Every sixth death in the world is due to cancer and it is the second leading cause of death, after cardiovascular diseases (Ferlay et al., 2021). In 2020, there were around 10 million deaths globally from cancer (Naghavi et al., 2021). It has been estimated that the global annual number of new cancer cases will be 29.5 million and number of deaths from cancer will be 16.4 million by 2040 ("Cancer Statistics", 2021). Undoubtedly these figures are alarming and the entire scientific community has been trying for decades to come up with ways to treat cancer in more efficient and effective ways.

Unlike most other diseases, there is no cure for cancer. This is because even after symptoms have gone away, they may surface again after some months or years. Unfortunately, such is the feature of malignant cells. So instead, doctors like to use the term 'remission' to describe when signs and symptoms have been gone for a considerable period of time. In contrast to 'cure' where the disease will never come back, being 'cured of cancer' means not having the symptoms for a significant amount of time. Sadly, it does not guarantee that the cancer will never come back ("Is there a cure for cancer?", 2021).

Thus, novel approaches are continuously being innovated to treat cancer better which will allow people to be 'cured of cancer'. The most common and traditional ways often pose adverse side effects. One of the oldest and most common forms of treatment for cancer, chemotherapy, has multiple side effects. The main reason behind these undesirable side effects of chemotherapy arise because chemotherapy acts on all active cells of the body. Active cells are the ones that grow and divide. Cancer cells are active and so are all other healthy cells of the body. Thus, chemotherapy fails to be specific for cancer cells only ("Side Effects of

Chemotherapy", 2021.) Side effects of chemotherapy can be short-term and long-term. Short-term side effects are usually experienced while the treatment is ongoing. On the other hand, the long-term ones may arise months to years after treatment is completed. Some of the most common short-term side effects include fatigue, pain in different parts of the body, mouth and throat sores, appetite and hair loss, diarrhea, nausea, constipation, nerve damage and blood disorders like anemia, leukopenia and thrombocytopenia. Long-term side effects include permanent damage to the heart, lung, liver, kidneys, or reproductive system ("Side Effects of Chemotherapy", 2021) (Partridge, Burstein & Winer, 2001). Radiotherapy also is non-specific and affects cells near the cancer cells. While the side-effects vary from the type of cancer and location, most are similar to those of chemotherapy ("Radiation Therapy Side Effects", 2021). That is why new methods and modes of treatment are being researched and brought about by scientists and health experts. One such ingenious approach is the use of our immune system for cancer treatment. This is called immunotherapy as humans' own line of defense, the immune system, is being used in this therapy.

The immune system of humans is naturally able to detect tumor antigens and bring about responses against them. Immunotherapy, also known as immuno-oncology, works by enhancing the natural ability of the components of the immune system so that they are able to function better and be more effective against cancer cells ("Immunotherapy for Cancer", 2021) (Rescigno et al., 2021). The earliest incident of using immunotherapy for treating cancer can be traced back to 1891, when William Colley (known as father of immunotherapy) saw that a mixture of live and inactivated *Streptococcus pyogenes* and *Serratia marcescens* were able to cause tumor regression in sarcoma patients (Zhang & Zhang, 2021). Ever since then, work is being done to harness the immune system in various ways to fight cancer better. Immunotherapy works by enabling immune cells to recognize and attack specific cancer cells, boost activity of existing immune cells towards tumor cells or by providing additional immune

components into the body which will act against cancer cells and thus enhance immune response. Immunotherapy is one form of biotherapy as it utilizes materials from living organisms to treat diseases. What makes this form of treatment promising is that it targets and attacks cancer cells only, without affecting healthy cells. Moreover, the immune system is dynamic and can adapt to changes quite fast. This feature is very advantageous when treating cancer because if a tumor is able to escape recognition, the immune system can find out ways to attack it again. The 'memory' of the immune system recognize treated cancer cells and can attack them if they return ("What is Immunotherapy?", 2021). Immunotherapy has proven to be a promising form of cancer treatment through various research and clinical trials. It has shown to have significant implications in fighting cancer. Different components of the immune system are being used for the purpose.



## **Chapter 2**

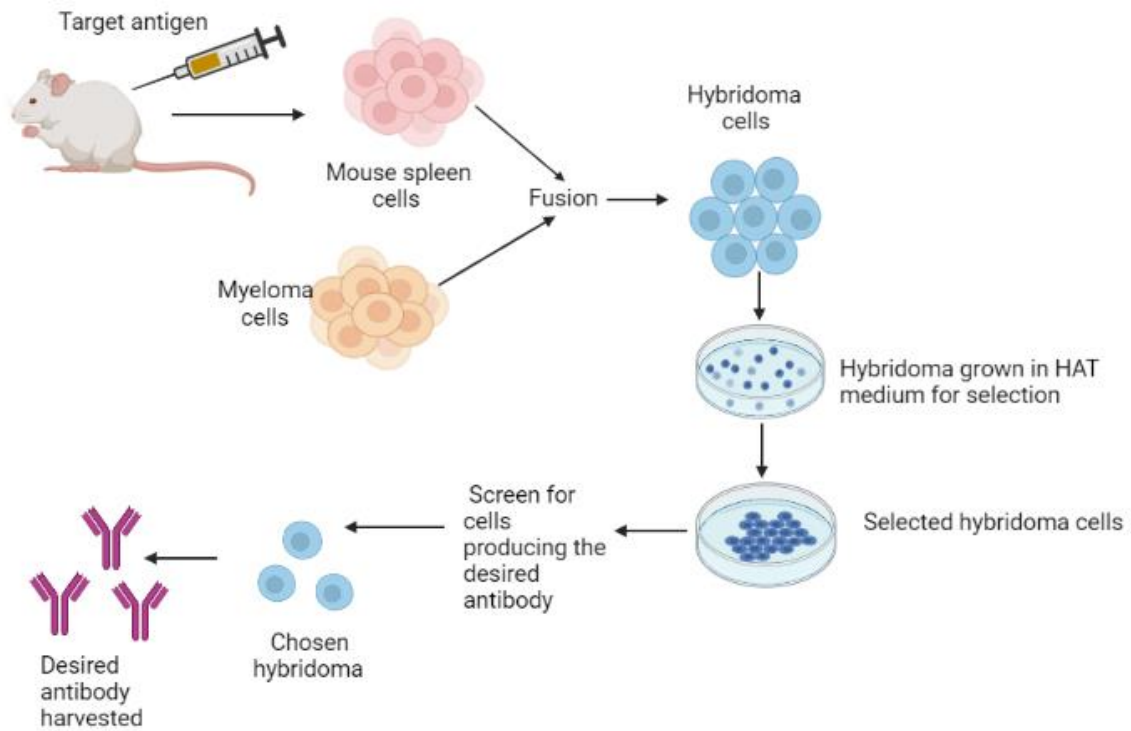
### **Different immunotherapies for cancer treatment**

Immunotherapy can be classified according to the component being used. Some of the most promising and recent immunotherapy choices have been discussed below.

#### **2.1 Monoclonal Antibodies**

Monoclonal antibodies (mAbs) are antibodies that are produced in vitro. These laboratory-made antibodies are designed to target a specific antigen. As these antibodies are able to bind specifically to a particular antigen in the human body, they can be used to target cancer/tumor antigens. Some mAbs directly attack cancer cells and this is referred to as target therapy. Others do not directly kill cancer cells but assist the immune system in being more effective in tracing cancer cells. In short, these mAbs enhance responsiveness of our immune system towards cancer cells ("Monoclonal Antibodies and Their Side Effects", 2021).

Monoclonal antibodies are made from hybridomas. Hybridoma is a hybrid cell line which is a result of fusion between spleen and myeloma cells. The hybridoma has the ability to continuously produce identical antibodies. The process starts by injecting a mouse with target antigen, e.g., antigen present on lung cancer cells. Spleen cells of the mouse will produce B-cells that are specific for that particular antigen. These spleen cells are extracted from the mouse and fused with myeloma cells to generate the hybridoma. The purpose of the hybridoma is to acquire a cell line that has the ability to produce specific antibodies (which comes from the spleen cells) and can grow indefinitely (from myeloma cells) (Nelson, 2000) (Sevier et al., 1981) (Pontecorvo, Riddle & Hales, 1977). The fusion of the two cells mostly happens via chemical method, in the presence of polyethylene glycol. These fused cells are then grown in HAT (hypoxanthine-aminopterin-thymidine) medium for 10-14 days. The HAT medium will kill off any unfused myeloma cells and ensure only hybrid cells are present (Korbakis et al., 2015). The next step is to identify whether these hybridoma are producing the antibody of interest, for which different techniques can be used like ELISA, Immunoperoxidase procedures, etc. (Lansdorp, Astaldi, Oosterhof, Janssen & Zeijlemaker, 1980) (Nelson, 2000). After the hybridoma are identified, they are grown in media like RPMI-1640 (Hellstrom & Hellstrom, 2014) and the antibodies harvested. Figure 1 demonstrates monoclonal antibody production.



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Figure 1 Monoclonal Antibody production

As of 2011, eleven mAbs were approved by FDA to be used as anticancer agents and this number increased twice as much by 2015 (Alewine, Hassan & Pastan, 2015). There are various mechanisms of action of mAbs.

One feature of tumor cells is that they have increased growth factor receptors on their surface. Some monoclonal antibodies either prevent growth factors from binding to the tumor's receptors, or damage the receptor function. The end result is the same- hindered growth of the tumor cells and consequent apoptosis. One such example is the first ever approved mAb, Rituximab. Erbitux is a mAb used in colorectal cancer. It interferes with the interaction between the malignant cells' epidermal growth factor receptor (EGFR) and the respective ligand.

HER2 (human epidermal growth factor 2) is a growth factor receptor overexpressed in breast and ovarian cancers. mAbs like Herceptin function by hindering dimerization and internalization of the receptor, both of which are needed for successful binding to growth factor.

Certain mAbs work by utilizing the immune system's inherent activities. These mAbs are mostly modified by, e.g., defucosylation of their oligosaccharides. These modified mAbs are better able to induce complement-dependent-cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) against tumors than normal antibodies in the body. The increased CDC and ADCC against tumor cells ensure that more cancer cells are killed.

A striking feature of many tumor cells is that they produce vascular endothelial growth factor(VEGF) which bind to their respective receptors on vascular endothelium. This results in the formation of new blood vessels, known as angiogenesis. Angiogenesis helps in metastasis. Avastin is one such mAb that works by inhibiting binding of VEGF with endothelium and thus prevents tumors from migrating from its site of origin to other parts of the body.

mAbs can be conjugated chemically with cancer drugs to increase their therapeutic efficacy. The tumor-binding capacity of the mAb helps transport the drug bound to it towards the target tumor. Brentuximabvedotin is an immunoconjugate comprising of anti-CD30 mAb with the drug monomethyl auristatin E to treat relapsed Hodgkin lymphoma or anaplastic large cell lymphoma.

Then there are mAbs that directly bind to antigen on tumors to cause a series of activities inside the tumor cells which eventually causes the tumors to self-destruct (Zahavi & Weiner, 2020) (Alewine, Hassan & Pastan, 2015).

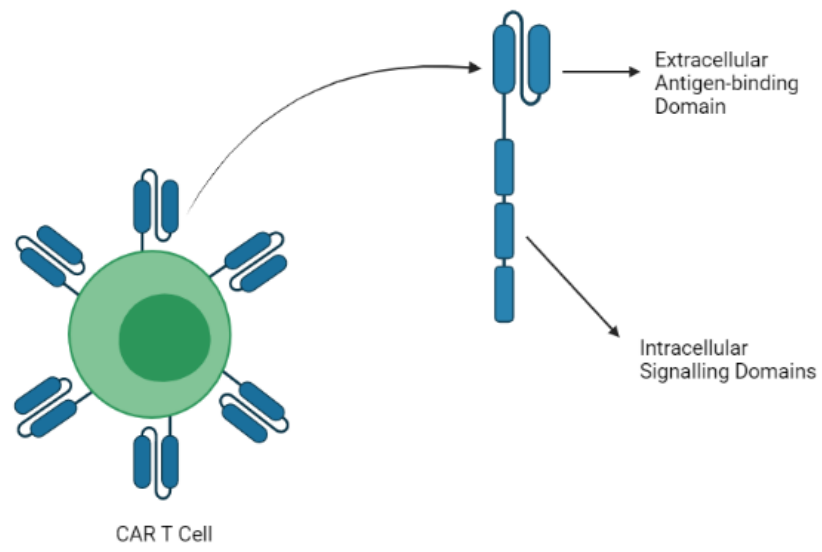
Monoclonal antibodies do have certain side effects. As these are proteins themselves, they may induce allergic reactions. The side effects may range from mild skin rashes at site of injection, to influenza-like symptoms, acute anaphylaxis, to even the threatening systemic inflammatory

response syndrome. TLS (Tumor Lysis Syndrome) and drug-induced immune thrombocytopenia have been linked with mAbs (Hansel, Kropshofer, Singer, Mitchell & George, 2010).

## **2.2 CAR (Chimeric Antigen Receptor) T cells**

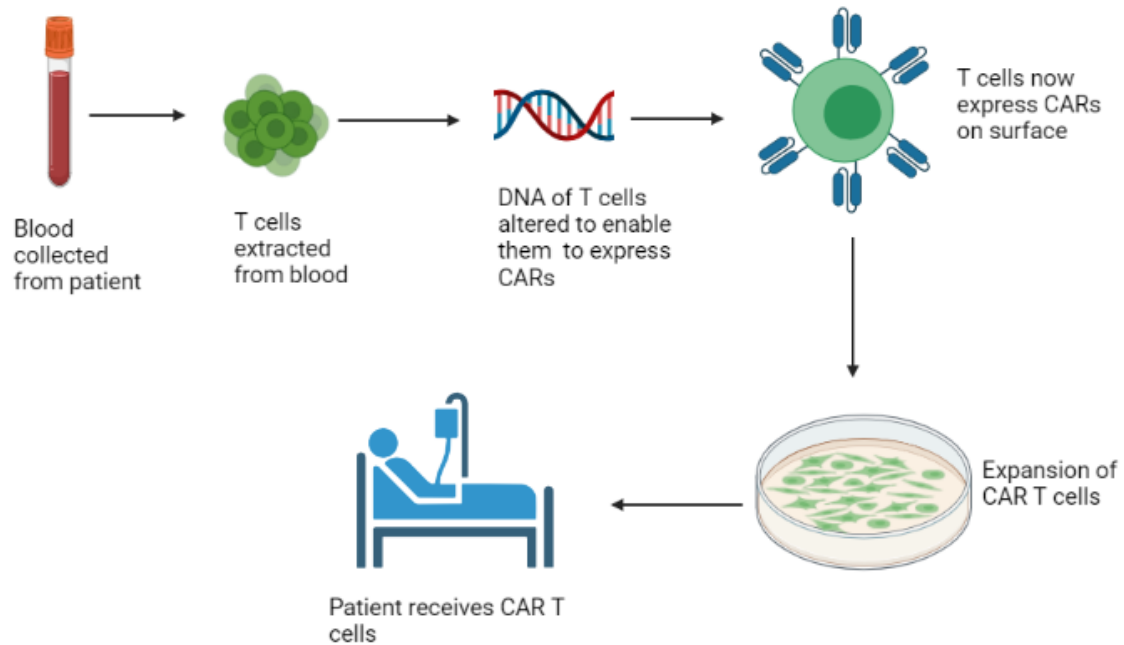
CAR T cells are T cells that have been modified by the addition of receptors on their surface which allows them to bind to tumor antigens and kill them.

Blood from patient is withdrawn by apheresis and T cells (both CD4+ and CD8+) extracted. Rest of the blood is returned back. These T cells are genetically engineered. In most cases, a retroviral vector is used to carry the gene of interest to the T cells. This gene of interest codes for receptors complementary to a target tumor antigen. The recombinant T cells now express the desired receptors on their surface and are known as CAR T cells. The CAR T cells are grown in in the laboratory to increase their numbers. Patient then receives an infusion of the CAR T cells ("Chimeric Antigen Receptor (CAR) T-Cell Therapy | Leukemia and Lymphoma Society", 2021). The term chimeric is used for the receptors because they have two properties. They have an extracellular domain mostly derived from the variable region of antibodies, which allows the receptors to bind to target antigen (Figure 2). Their intracellular signaling and costimulatory domain derived from T cells enable them to be activated upon binding (Martínez Bedoya, Dutoit & Migliorini, 2021). Figure 3 displays CAR T cell therapy.



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*Figure 2 CAR T cell*



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*Figure 3 CAR T cell therapy*

CAR T cells perform in different ways to kill the target cancer cells. The CAR T cells secrete perforin and cytotoxic granules by exocytosis. The perforin creates holes on the plasma membrane of the target cancer cell, allowing the granules to enter their cytoplasm. The granzymes in the granules cleave substrates which are very important for the lifespan of the cancer cells thus leading to their apoptosis (Benmebarek et al., 2019) (Cullen & Martin, 2007).

CAR T cells can utilize the Fas and FasL pathway to kill tumor cells, and is used mostly for cancers of blood. Fas is a receptor that causes programmed cell death after it has bound to its ligand, FasL. Certain CAR T cells can be engineered to contain surface Fas ligand that are complementary to Fas on cancer cells. After FasL of CAR T cells bind to Fas on cancer cells, apoptosis of the cancer cells occurs (Waring & Müllbacher, 1999) (Walczak, 2013). This is a

very good mechanism of tumor killing for those cancer cells that do not express any antigen on their surface.

Some CAR T cells secrete cytokines which upregulate interferon gamma (IFN- $\gamma$ ) receptors on tumor stroma. The increased interferon receptors induce an immune response against them (Textor et al., 2014).

There are five FDA- approved CAR T cell therapies involved in treating B cell lymphoma, multiple myeloma, lymphoblastic leukemia, etc. (Whilding & Maher, 2015) ("FDA-approved CAR T-cell Therapies | UPMC Hillman", 2021). Many more are under pre-clinical and clinical trials to be soon used as therapeutics.

The side effects of CAR T cells are mainly related to toxicity. Cytokine Release Syndrome (CSR) is one of the most common threatening results of this type of therapy (Maude, Barrett, Teachey & Grupp, 2014). Dendritic cells (DCs), macrophages and lymphocytes are overactive in CSR. Neurotoxicity has also been seen in some cases after CAR T cell treatment, leading to visual hallucination, dysplasia and epileptic seizures (Davila et al., 2014).

## **2.3 Cytokines**

Cytokines are polypeptides or glycoproteins produced by a number of cells, mostly by helper T cells and macrophages. Their functions vary from cell proliferation, differentiation, causing inflammation, inducing immune response, etc. Cytokines bind to receptors on target cells, leading to intracellular signaling and thus bringing about the desired effect (Berraondo et al., 2018). Cytokines are important components of the immune system and have anti-cancer properties as well. Some have direct effects like preventing proliferation of cancer cells and bringing about apoptosis. Others perform indirectly by triggering the other members of the immune system to act against cancer cells. Interleukin 2 (IL-2) and Interferon alpha (IFN- $\alpha$ ) are the two cytokines approved by FDA to be used as cancer treatment. IL-2 is used for treating



advanced renal cell carcinoma and metastatic melanoma while IFN- $\alpha$  for hairy cell leukemia, follicular non-Hodgkin lymphoma, melanoma and AIDS-related Kaposi's sarcoma (Berraondo et al., 2018).

IL-2 causes proliferation of Natural Killer (NK) cells and T lymphocytes. An infusion of this cytokine results in increased amount of NK and lymphocytes which in turn means more cells are available for acting against tumor cells (Waldmann, 2017). A high dose of IL-2 was given to patients with metastatic renal carcinoma and clinical responses were observed, with 5% complete response and 9% partial response. These responses were seen to persist for long periods after the therapy (ROSENBERG et al., 1989). However, using cytokines in high dose can result in threatening issues like various toxicities, hypotension and capillary leak syndrome (Dutcher et al., 2014). To avoid these, cytokines are used in combination with other immunotherapies instead of being used alone, or they undergo certain modifications. IL-2 receptors may contain the IL-2R $\alpha$  chain only or both IL-2R $\alpha$  and IL-2R $\beta$  chains. Regulatory T cells express IL-2R $\alpha$  in large amounts and thus infused IL-2 ends up binding with these instead of NK cells or T lymphocytes (Waldmann, 2017). IL-2 have been engineered so that they bind to IL-2R $\beta$  chain only and not with IL-2R $\alpha$  to ensure that the infused IL-2 will bind to NK cells and T lymphocytes as these have anti-tumor activities. This changed mechanism of IL-2 ensures that they will not bind and get utilized by regulatory T cells, as they have no part to play in killing cancer cells. The modified cytokine NKTR-214 is one such example which has shown significant positive results in clinical trials (Charych et al., 2017).

IL-15 leads to ontogenesis and proliferation of both NK cells and CD8+, induces their cytotoxicity and causes them to release other cytokines (Lodolce et al., 1998). It has an advantage over IL-2 because it does not bind to regulatory T cells, which means that infused IL-2 will act on desired cells like NK cells, only (Marshall, Sinclair, Tung & Seddon, 2014). Recombinant aglycosylated IL-15 were given to patients with advanced melanoma and renal

cell carcinoma. There was a significant increase in patients' NK cells and CD8+ cells and 5 out of 18 patients had 10-30 % reductions in their marker lesions. The trial could not proceed unfortunately because of side effects like hypotension and thrombocytopenia (Conlon et al., 2015). Many other interleukins like IL-12,10,21, are undergoing clinical trials to be used as monotherapy for cancer, or combined with other drugs.

Interferons are another type of cytokines with multiple anti-cancer functions. They increase MHC (major histocompatibility complex) expression on tumor cells, induce apoptosis of cancer cells and prevent angiogenesis (Waldmann, 2017). IFN- $\alpha$  treatment for hairy cell leukemia resulted in improved platelet and hemoglobin levels (Ratain et al., 1985). A randomized control trial divided follicular lymphoma patients into two groups. One received CHVP chemotherapy and the other received CHVP therapy with IFN- $\alpha$ . The group getting the combination therapy showed longer progression-free survival (Waldmann, 2017). Similar results have been found after IFN- $\alpha$  treatment for AIDS-Related Kaposi Sarcoma and malignant melanoma.

## **2.4 Immunomodulators**

Immunomodulators are compounds with the ability to interact with the immune system and cause it to function in a certain way. These modulators can be endogenous which are produced by the body itself, like cytokines. They can also come from outside sources like food and are thus exogenous. Many food items like fruits and vegetables are known to increase the number of cells of the immune system. Immunomodulators act on components of the immune system, like NK cells and other lymphocytes to either suppress or enhance their actions ("What are immunomodulators and how do they work? - KHNI", 2021). In the light of immunotherapy for cancer, immunomodulators like cytokines are used to stimulate T and NK cells to increase their activity against malignant cells. Many of the approved immunomodulatory therapies for cancer

release interleukins and interferons in the body, that will activate NK and cytotoxic T cells. Thalidomide, lenalidomide, and pomalidomide are immunomodulatory drugs that release IL-2 and are used for treating multiple myeloma (Matsushita & Kawaguchi, 2018). The administration of such drugs may often cause over activate the immune system, leading to side effects like fever, flu-like symptoms, neurotoxicity and other serious allergic reactions (Bascones-Martinez, Mattila, Gomez-Font & Meurman, 2014). To avoid such occurrences, more specific immunomodulators are have promising implications, that will activate immune cells only at desired locations. Such a step prevents NK and T cells in all parts of the body to be stimulated and thus stops the immune system to be over active. The use of stimuli-responsive nano immunomodulators is one such example (Li et al., 2021). These nano-immunomodulators utilize properties of the microenvironment of tumors to act very specifically. The microenvironment of tumors has certain characteristics. It is more acidic than environment of normal tissues due to the tumor's increased ability to take part in glycolysis and produce lactic acid. Cancer cells also overexpress reactive oxygen species (ROS), glutathione and enzymes like matrix metalloproteinases (MMPs) and hyaluronidase (HAase). All these properties make the microenvironment of cancer cells different from normal tissues. The nanomaterials are used as carriers to deliver a drug, e.g., a dose of cytokine, to the tumor site only. These nanocarriers become active only when they have reached a territory which has certain features, like high acidity or high concentration of ROS of tumor microenvironment. The delivery of cytokine at tumor site only can prevent undesirable side effects like a hyperactive immune system (Li et al., 2021).

Immunomodulatory drugs like Azacitidine and Decitabine act in a different way. Hypermethylation in promoter of tumor suppressor genes cause them to be silenced. Hypomethylating agents (HMAs) are analogues of pyrimidine nucleosides which get incorporated into a tumor suppressor gene's DNA and inhibit the methylation at the promoter.

Thus the genes are no longer silenced. HMAs like Azacitidine and Decitabine have been approved to treat myelodysplastic syndromes and acute myeloid leukemia (Derissen, Beijnen & Schellens, 2013) (Schroeder, Rautenberg, Haas & Kobbe, 2016). HMAs also increase expression of cancer-specific antigens and MHC on surface of cancer cells. As a result, they are more readily 'noticed' by cells of the immune system and faster response is brought about (Srivastava et al., 2016).

## **2.5 Immune Checkpoint Inhibitors**

Immune checkpoints is a collective name given to certain ligand-receptor interaction that are involved in regulating the immune system. These checkpoints are responsible for self-tolerance and maintaining immune homeostasis. They also determine the extent of immune reaction to prevent tissue damage and protect normal cells from coming under attack by immune cells (Fan, Geng, Shen & Zhang, 2020). Most of the receptors are present on T cells which are activated after binding to their respective ligands. The most common checkpoint receptors on T cells are LAG-3, PD-1 and CTLA-4. All three of them prevent over expansion of T cells that would lead to a hyperactive immune system ("Immune Checkpoints Role in Immunity & Cancer (Mini-review) | Bio-Rad", 2021). An over active immune system may lead to fatal side effects and autoimmune diseases and immune checkpoints play a crucial role in preventing such occurrences. When checkpoints are present on T cells, they are mostly referred to as receptors. They may be present on other cell types too. Some tumor cells express them on their surface and are mostly termed as checkpoint ligands (Marcucci, Rumio & Corti, 2017). The presence of such commodities on tumor cells have proven to be quite advantageous for them. Checkpoint ligands cause loss of cell adhesion thereby allowing cancer cells to metastasize. Moreover, these cancer cells are able to evade apoptosis (Polyak & Weinberg, 2009).

The checkpoint ligands on surface of tumor cells bind to checkpoint receptors on T cells. This binding activates the inhibitory effects of the checkpoint receptors and eventually stops functions of T cells. This is how checkpoints play a part in tumor immune evasion (Webb et al., 2018). Anti-CTLA-4 and anti PD-1 drugs have been approved to be used against cancers. These work by inhibiting the function of checkpoint receptors on T cells. Consequently, the activities of T cells continue without any stoppage (Sharpe, 2017). The anti-CTLA-4 drug Ipilimumab has been approved by FDA for treating melanoma (Schadendorf et al., 2015). Anti-PD-1 drugs like Pembrolizumab are used for lung cancer, kidney, head, neck, and bladder cancer (Sharpe, 2017).

The checkpoint inhibitors are mostly monoclonal antibodies that specifically bind to checkpoint receptors on T cells, thus preventing them from binding to their corresponding ligands. In clinical trials, Ipilimumab showed significant results. It not only increased survival of patients with metastatic melanoma, but enhanced durability of response to treatment. About 15-20 % of people treated with it had long-term control of their disease (Hodi et al., 2010). The average duration of response in patients receiving Ipilimumab was 2 years, while that of chemo and oncogene-related therapies was only 4 to 8 months (Thompson et al., 2012). mAbs that target the PD-1 pathway also showed satisfactory results. Response rates between 20 to 50% were found with PD-1 targeting agents for different cancers (Topalian et al., 2012). Patients not responding to anti-CTLA-4 mAb treatment were recommended Pembrolizumab and Nivolumab by FDA to be used to treat advanced melanoma (Ascierto & Marincola, 2015).

Most of the side effects of immune checkpoint inhibitors are associated with disrupted homeostasis of the immune system. T cells are always having to remain active without any shut-off signal. This may lead to symptoms like diarrhea, skin rashes breathing difficulties. More serious cases may lead to autoimmune disease like Rheumatoid arthritis. About 10 % of patients receiving this kind of therapy develop autoimmune reactions (West, 2021).

## 2.6 Oncolytic Viruses

Oncolytic viruses are attenuated viruses designed to infect tumor cells and bring about an immune reaction against them (Orange, Reuter & Hobohm, 2016). A variety of viral species are being investigated to be used as potential oncolytic viruses, especially Herpes viruses (Russell, Peng & Bell, 2012) (Liu et al., 2003). Currently, the only FDA approved oncolytic viral therapy is a genetically modified Herpes virus, talimogene laherparepvec (T-Vec or Imlygic), for treating metastatic melanoma. Many more are under pre-clinical and clinical trials and their use is not limited to melanomas only but extends to pancreatic and hepatocellular carcinomas. The most striking advantage of oncolytic viruses over other therapies is its high tumor tropism and the fact that its activity is independent of any receptor or ligand (Raja, Ludwig, Gettinger, Schalper & Kim, 2018). The virus is genetically altered to remove any virulence factor to ensure its safety before injection into the body. The virus has high tropism for tumor cells and will thus infect them only. Pathogen Associated Molecular Patterns (PAMPs), which may be the viral capsid, genome or other proteins, leads to an anti-viral immune response. The genetic material of the virus activates protein kinase R. This kinase stops viral mRNA translation. As a result, viral protein synthesis is stopped after some time which eventually leads to apoptosis of the viral-infected tumor cells. The virus-infected tumor cells lyse as a result, release many compounds in the tumor microenvironment. Some of these components are already-replicated viral particles which will go on to infect remaining tumor cells. Others include viral genome, interferons, chemokines and danger-associated molecular patterns (DAMPs) like HMGB-1, calreticulin. All of these released compounds recruit immune cells like phagocytes, to the tumor site (Kaufman, Kohlhapp & Zloza, 2015) (Kohlhapp & Kaufman, 2015).

Oncolytic viruses like T-Vec are modified in other ways too to bring about anti-tumor activities. One example is the elimination of the viral ICP47 gene and insertion of the human GM-CSF gene. ICP47 directly associates with the transporter involved with antigen-presentation and processing, thus reducing viral antigen presentation. Consequently, antigens are loaded less onto MHC I. As the modified virus lack ICP47, the infected cancer cells have enhanced antigen-presentation. Moreover, the incorporated GM-CSF gene codes for the GM-CSF regulatory cytokine that causes dendritic cell accumulation and mediates T cell responses (Tomazin et al., 1998).

Side effects depend on the virus used, the type of cancer treated, location of tumor, patient's overall health, etc. The most common side effects arise due to hyperactivity of immune cells and may cause nausea, chills, fever, and pain at injection site ("Oncolytic Virus Therapy", 2021).

## **2.7 Cancer Vaccines**

Cancer vaccines can be of two types. One is the conventional kind which is used to prevent cancer, especially those caused by viruses. These include the Human Papilloma Virus (HPV) vaccine to prevent cervical cancer and Hepatitis B vaccine to prevent Hepatitis B (Babu, Kumar, Reddy & Anuradha, 2010). The other type is a form of immunotherapy and is termed as treatment/therapeutic cancer vaccine. Therapeutic cancer vaccines activate immune system against a tumor antigen that the body has already been exposed to. Instead of preventing future occurrence of a cancer, these vaccines are used to treat it (Pardoll, 1998). Different events or circumstances determine whether a tumor will activate immune cells or evade an immune response. Whether a tumor antigen will escape immunosurveillance depends on who is presenting it to the immune system and how. It has been seen that if the presence of any foreign antigen leads to tissue destruction or inflammation (in most cases of viral or bacterial infection),

the result is mostly T cell activation. On the other hand, if an antigen does not lead to inflammatory reactions or tissue damage (as seen in tumor antigens), that may lead to tolerance. When inflammatory cytokines are released, Antigen Presenting Cells (APCs) produce co-stimulatory molecules like B7 which cause T cell activation. But in the absence of co-stimulatory molecules, ignorance, anergy or apoptosis of the antigen specific T-cells may occur. One of the primary goals of therapeutic cancer vaccines is to counteract tolerance of T cells to tumor antigens. Studies have demonstrated that antigens endocytosed by APCs derived from bone marrow are loaded onto both MHC I and II pathways. Cancer vaccines target tumor antigens to these bone marrow-derived APCs (Pardoll, 1998).

Therapeutic cancer vaccines often contain the tumor cells themselves that will initiate an immune response against them and also on other similar already-existing antigens in the body. Some of these vaccines can be mixed with adjuvants like adjuvants such as Bacillus Calmette Guerin (BCG) and Corynebacterium parvum, to further stimulate the immune system (Berd, Maguire, McCue & Mastrangelo, 1990). Another approach transduces tumor cells with viral and allogenic MHC genes to increase their immunogenicity. At times, the tumor cells are genetically modified to contain genes expressing cytokines and co-stimulatory molecules (Pardoll, 1995). The local release of cytokines prevents toxicity. The most commonly used cytokine gene is granulocyte-macrophage colony stimulating factor (GM-CSF) promotes local dendritic cell differentiation at the vaccination site. Dendritic cells differ from other APCs like granulocytes and macrophages and are capable of bringing about very strong T cell responses (Banchereau & Steinman, 1998).

Cancer vaccines can also contain the tumor antigen only instead of whole cells. These tumors are mostly the ones that have been constantly evading immune response inside the human body (Pardoll, 1998). Recombinant viral vaccines have promising implications as well. These work by incorporating the tumor antigen gene into viral genome. The attenuated and modified virus



acts as carrier for the antigen of interest. Cellular damage caused by the viral infection releases danger signals like cytokines which attract bone marrow-derived APCs. Another mode of action of viral vaccines is to infect the marrow-derived APCs directly. This enables better processing of the encoded antigens in MHC I pathway (Minev et al., 1994).

Dendritic cells (DCs) are also used in cancer treatment vaccines as they have great potential in activating T cells to carry out anti-tumor activities. DCs express 50 times more MHC molecules than macrophages and produce adhesion and co-stimulatory molecules needed to activate T cells. The DCs are used as antigen carriers and different forms of antigen like peptide to whole proteins, can be loaded onto DCs (Pardoll, 1998).

Some of the FDA- approved therapeutic vaccines include TheraCys for urothelial carcinoma, sipuleucel-T (Provenge®) for prostate cancer and T-VEC for advanced melanoma (Rousseau, Hirschmann-Jax, Takahashi & Brenner, 2001). Just like any other vaccine, side effects of cancer vaccines include headache, mild fever, chills, fatigue and nausea ("Cancer Vaccines and Their Side Effects", 2021).

## Chapter 3

### Challenges of Cancer Immunotherapy

Conventional cancer treatments like chemo and radio therapies have many undesirable side effects. Many of the effects arise because these treatments are often not specific and end up affecting other healthy body parts. Some of the most common side effects include hair loss, fatigue, nausea, diarrhea, loss of appetite and even infertility ("Chemotherapy Side Effects", 2021). Immunotherapy does not lead to many of these results, yet it is still not as widely used as it should be. Despite having promising outcomes, immunotherapy is not extensively used for treating cancers. This is because immunotherapy too, has certain shortcomings which are preventing its wide-scale use.

One of immunotherapy's major challenges is that its efficacy is low. Many immunotherapies have showed efficacy in limited types of cancers, and only a few patients having those cancers (Ventola, 2017). The variability of outcome in different patients may arise due to multiple factors like the need to identify additional biomarkers and cancer pathways, tumor heterogeneity, difference in cancer type and stage, treatment history, and the underlying immunosuppressive biology of the cancer (Ventola, 2017). One example of patient outcome variability is the response to immune checkpoint inhibitors, CTLA 4 and PD-1. Clinical trials have demonstrated that only 15% to 25% of patients with various types of cancer responded to the above mentioned checkpoint inhibitor therapy (Zugazagoitia et al., 2016). Another significant challenge is the identification of tumor-specific antigens (TSAs), also termed as neoantigens. TSAs are expressed by tumor cells only, in contrast to tumor-associated antigens (TAAs) which are expressed by both tumor and healthy cells. Due to lack of knowledge of known TSAs, many immunotherapies target TAAs, thus bringing about non-specificity in the treatments and associated toxicities (Schumacher & Schreiber, 2015).

There is a need for identification of more predictive biomarkers to enhance use of immunotherapy. Predictive biomarkers determine how a patient will respond to a certain treatment. Only a few biomarkers that have showed how patients may respond to a particular immunotherapy have been confirmed. For example, 20% of patients with gastric cancer have showed HER 2 amplification. These patients had response rates between 40%-50% after treatment with the monoclonal antibody Trastuzumab. (Zugazagoitia et al., 2016). Many clinical trials have provided evidence that the higher the PD-L1 expression by tumor, the better the response rate and survival rates with PD-1/PD-L1 ICB treatment (Pardoll, 2015). Patients with advanced melanoma have showed positive clinical results after treatment with the checkpoint inhibitor, Ipilimumab. These patients had serum markers such as C-reactive protein, lactate dehydrogenase, soluble CD25, and vascular endothelial growth factor (Yuan et al., 2016). More such results are needed to ensure efficacy of immunotherapies, but the identification of such biomarkers which have predictive value is a lengthy process. Mutations that give a tumor characteristic properties have clinical predictive values. Information of such mutations would be valuable for immunotherapies. Determining such mutations call for highly sensitive, multiplexed, comprehensive sequencing techniques, and classic genomic sequencing like Sanger sequencing often lack the necessary finesse (Zugazagoitia et al., 2016).

Another major obstacle faced by immunotherapy is tumor heterogeneity. Tumor heterogeneity occurs because cells within a tumor often show different morphological and phenotypical properties. As a result, immunotherapies cannot act against all the cells that make up a tumor (Zugazagoitia et al., 2016). Cancer signaling networks are remarkably flexible and adaptive, making cancer cells resistant to a number of drugs. Secondary genomic mutations in the drug target, reactivation of dormant cancer pathways and activation of alternative signaling pathways, are three mechanisms that lead to immunotherapy resistance in cancer cells (Camidge, Pao & Sequist, 2014). Patients with advanced melanoma who had developed acquired treatment resistance to the checkpoint inhibitor Pembrolizumab, were analyzed by taking tumor biopsies. Mutations in the JAK1 or JAK2 gene was found in two of the patients. These mutations caused a disruption in the IFN-gamma signaling pathway which reduced the expression of genes involved in T-cell recognition and elimination of cancer cells. Another patient showed a mutation in the B2M gene. This gene is crucial for expression of surface proteins on immune cells that recognize and kill cancer cells ("Mutations Linked to Immunotherapy Resistance", 2021).

Any kind of cancer treatment is expensive and immunotherapies are often costlier than traditional forms of treatment. A total of \$3.8 billion for malignant melanoma and \$83.9 billion for non-small- cell lung cancer were needed globally in a year for treatment with the checkpoint inhibitor Pembrolizumab. \$1.7 billion and \$47.2 billion were required for the two cancers respectively when Nivolumab was used (Tartari et al., 2016). The expense of immunotherapies is daunting for many and it often becomes next to impossible to bear the expenses for treatment. Not all countries provide health insurance and even in places where they do, the expense is still too much.

## **Chapter 4**

### **Scopes and future of Immunotherapy for Cancer**

Works are under way to overcome many of the challenges faced by immunotherapy. More TSAs have to be identified to act as target for immunotherapies in place of TAAs. In this context, Cancer Testis Antigens (CTAs) possess significant implications. CTAs are expressed on healthy male germs cells and also on tumor cells of multiple human cancers. CTAs are considered as neoantigens when expressed by cancer cells (Gjerstorff, Andersen & Ditzel, 2015) and thus serve as excellent anti-cancer therapy targets. Next-Generation Sequencing (NGS) should be used for better and efficient screening of clinical predictive genomic mutations that lead to cancers (Zugazagoitia et al., 2016). More predictive and prognostic biomarkers, cancer signaling pathways and oncogenic mutations are being identified. Many of these high-throughput research are being able to occur due to ventures by The Cancer Genome Atlas (funded by the National Cancer Institute and National Human Genome Research Institute) and the International Cancer Genome Consortium.

Drug combinations that have more than one target is seen as a potential improvement for immunotherapy. This can greatly improve efficacy as well. An even sophisticated approach is using the specific biomarkers or pathways that drive the biology of each patient's tumor, to give a drug combination therapy. This is more of a personalized form of immunotherapy.

As it is wisely said that prevention is better than cure, multiple works are being done to come up with effective preventive cancer vaccines. Most of these vaccines utilize antigens expressed by cancer cells only, and ‘ready’ our immune system to be equipped if such antigens ever arise in the body. Efforts are being put in finding out tumor antigens that are expressed on early cancers or premalignant lesions so that they can be administered to people with high risk of cancer (Finn & Beatty, 2016). Vaccines for prevention of non-virally induced cancers is only in its very primary stage of development and have a long way to go before reaching trials.

Table 1 demonstrates some success stories of using immunotherapy for treating cancer. Such stories encourage more work and research towards immunotherapy for cancer treatment.

Table 1 Success stories of Immunotherapy for cancer treatment

<b>Name of patient</b>	<b>Cancer type</b>	<b>Treatment</b>
Sharon Belvin	Melanoma	In 2004, at the age of 22, Sharon was diagnosed with stage 4 melanoma. Due to experiencing side –effects, Sharon was prescribed checkpoint blockade immunotherapy which was undergoing clinical trial. Ipilimumab (Yervoy), a CTLA-4 checkpoint inhibitor, was used. She was declared cancer-free one year later, after four rounds of treatment ("Patients", 2021).
Maureen O’Grady	Lung cancer	In 2009 after being diagnosed with stage 4 lung cancer, Maureen was given only about a year to live. After both chemo and targeted therapies failed, she decided to take part in a clinical trial of Nivolumab, a checkpoint inhibitor. The first scans showed excellent response in areas of disease while the second set of scans showed no new growth of the tumor ("Patients", 2021).
Judy Perkins	Breast cancer	In 2003, Judy was diagnosed with breast cancer, and ten years later the cancer returned as metastatic. She decided to take part in a clinical trial which used tumor infiltrating lymphocytes (TIL). Soon enough her tumors disintegrated fully and she became the first person to be cured of metastatic breast cancer through TIL therapy ("Patients", 2021).
Jean	Advanced non-Hodgkin lymphoma	Jean was diagnosed with the cancer in 2006. She took part in a trial that used the monoclonal antibody Rituximab. She became cancer-free afterwards ("Patient stories about immunotherapy", 2021).

## **Chapter 5**

### **Conclusion**

Cancer claims thousands of lives every year. As finding a definite cure for it remains next to impossible, the only way to fight it is through treatment. The commonly used conventional methods of treatment like chemo and radio therapies are often non-specific for cancer cells and end up affecting other body parts, leading to undesirable and often threatening side effects. The immune system of humans has inherent abilities to attack and destroy cancer cells. Immunotherapy utilizes this property of the immune system. Immunotherapy is being considered to have great potential as anti-cancer treatment as it harnesses the immune system and enhances its activity to destroy tumor cells. This therapy uses a variety of components of the immune system for its anti-tumor activities. There are already quite a lot of FDA- approved immunotherapies and many are in the last phase of clinical trials. Despite showing promising implications, immunotherapy is still not used widely. It faces multiple challenges and shortcomings. These challenges will have to be overcome if immunotherapy is to become a common mode of cancer treatment. With the effort of the entire scientific community and associated governing bodies, immunotherapy will undoubtedly emerge as an excellent choice for cancer treatment, with the ability to save many lives in the future.

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