IMMUNOTHERAPY IN THE MANAGEMENT OF LUNG CANCER

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

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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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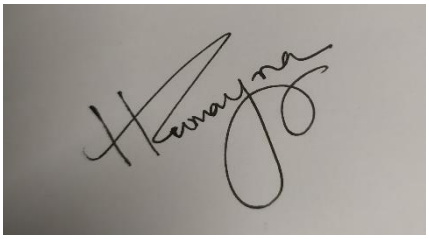

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Approval

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Ethics Statement

This study does not involve any human or animal trial.

Immunotherapy in the Management of Lung Cancer

Abstract

Among all the major cancer types, lung cancer is one of the most common as it holds a higher percentage of cancer related mortality and treating lung cancer is yet a challenge. Chemotherapy, radiation therapy, surgery are the most commonly used and traditional treatment options for lung cancer but these possess side effects and various complications. Therefore, to minimize the side effects, a novel treatment option is needed and it is cancer immunotherapy. Immunotherapy is a new way to treat cancer that can attack tumor cells by using the host's immune system. Various immunotherapy drugs have been evaluated for lung cancer. This review focused on the relation between cancer and immune system, new immunotherapy treatment options for lung cancer as well as the challenges and prospects of cancer immunotherapy.

Keywords: Lung cancer, Immunotherapy, Cytokines, Checkpoint inhibitors, Adoptive cell therapy, Vaccines

Dedication

Dedicated to my lovely parents, teachers and friends

Acknowledgement

First and foremost, praises and thanks to the God, the Almighty Allah, for blessing me with good health and giving me patience, sincerity, knowledge to complete this project successfully.

I am extremely grateful to my parents for their prayers, love, care and sacrifices for educating and preparing me for my future. I owe my heartiest gratitude to the most undeniable person of my life, my mother, for her constant support which motivated me the most throughout my project work.

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List of Acronyms

ADC	Adenocarcinoma
APCs	Antigen presenting cells
ALKT	Anaplastic lymphoma kinase tumor
CAR-T	Chimeric antigen receptor T
CIK	Cytokine induced killer cells
CNS	Central nervous system
CTLs	Cytotoxic T lymphocytes
DC	Dendritic cells
DFS	Disease free survival
DFI	Disease free interval
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGGCT	Extragonadal germ cell tumor
EMA	European Medicines Agency
EPP	Extrapleural pneumonectomy
FDA	Food and drug Administration
GM-CSF	Granulocyte-macrophage colony-stimulating factor

IDO	Indoleamine-2,3-dioxygenase
IL	Interleukins
INF	Interferons
irPFS	Immune-related progression-free survival
LAK	Lymphokine activated killer cells
LCNEC	Large cell neuroendocrine carcinoma
MHC	Major histocompatibility complex
MAGE	Melanoma antigen
MDSCs	Myeloid-derived suppressor cells
NSCLC	Non-small cell lung carcinoma
NKTs	Natural killer T cells
OS	Overall survival
PSC	Pulmonary sarcomatoid carcinoma
PSCA	Prostate stem cell antigen
SCC	Squamous cell carcinoma
SCLC	Small cell lung carcinoma
TAAs	Tumor associated antigens
TCR	T cell receptor
TGF-2	Transforming growth factor-beta 2
Th	T helper

TIL	Tumor-infiltrating lymphocyte
TME	Tumor microenvironment
TNF	Tissue necrosis factor
TRT	Thoracic radiation therapy
TSAs	Tumor-specific antigens
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background

Lung cancer is the most commonly diagnosed cancer and it is the major cause of cancer related deaths. Its treatment is still challenging. Formerly, immunotherapy has failed to treat lung cancer but lately immunotherapy has been developed as an efficacious treatment for lung cancer which is a significant global concern in the cancer field (Steven et al., 2016). Immunotherapeutics can be stated as an extensive category of treatments aiming to express immune-mediated demolition of cancer cells (Massarelli et al., 2014). Cancer immunotherapy is a kind of cancer treatment that works by boosting the body's natural anti-cancer defenses (Aldarouish & Wang, 2016). Dr. William B. Coley, an American bone carcinoma surgeon, explained the principle of immunotherapy to treat cancer more than a century ago. He treated different malignant conditions with a streptococcal vaccine which became known as ‘'Coley's Toxins’ that included toxins from *Streptococcus pyogenes* and *Serratia marcescens*. He eventually noticed that patients with inoperable soft tissue sarcomas had the best response to “Coley’s Toxins” with nearly 50 % of patients achieving long-term (greater than 5 years) disease-free survival (Steven et al., 2016). There are two types of immunotherapy: active immunotherapy and passive immunotherapy. Passive immunotherapy refers to the use, in regard to drugs that directly target tumors, for example, monoclonal antibodies or adoptive cell therapy. On the contrary, the main aim of active immunotherapy is involved with boosting the patient’s immune system in order to destroy tumor by using tumor antigen vaccination, and non-specific immunomodulation with the use of bacterial products or targeting negative regulatory receptors that can prevent the immune response of tumor from developing (Aldarouish & Wang, 2016). Despite the potential of chemotherapeutic drugs to improve survival rate of cancer patients, there always remains a chance of cancer relapse even after receiving chemotherapy and health condition often deteriorates due to the severe side effects triggered from its non-target specific activity including weakening of the body’s natural immune responses (Aldarouish & Wang, 2016). Various immunotherapy treatments such as immune checkpoint inhibitors, vaccines, immunomodulators,

adoptive cell therapy and engineered T cell therapy shows promising results in lung cancer treatment by blocking the signals of immune checkpoint inhibitor on activated T cells and tumor cells and therefore, currently serves as an efficient treatment strategy against lung cancer alone or in combination with other therapeutic interventions (Steven et al., 2016).

1.2 Objectives of the Study

The objectives of this study are-

- to provide an overview of the concept of cancer and cancer immunosurveillance
- to provide an insight on the importance of immunotherapy in the management of lung cancer
- to review the various immunotherapeutic strategies available for lung cancer
- to analyze the responses to immunotherapy in clinical trials
- to identify the challenges and future prospects associated to cancer immunotherapy

1.3 Rationale of the Study

Immunotherapy is a treatment strategy that holds great potential to train the immune system in order to fight cancer. It is a novel and promising treatment approach for the patients with advanced lung cancer. To get a clear understanding of cancer immunotherapy, this review provides an overview about the concept of cancer, its immunosurveillance and the importance of immunotherapy in the management of lung cancer. Nowadays, multiple immunotherapy options are available for lung cancer treatment such as vaccines, checkpoint inhibitors, engineered T cell therapy and adoptive cellular therapy which demonstrated promising responses in clinical trials. Therefore, this review was conducted to help the reader to understand about the different immunotherapeutic strategies available for lung cancer and responses to this treatment modality in clinical trials, and also to discuss the challenges and prospects of cancer immunotherapy.

Chapter 2

Methodology

A comprehensive review based on immunotherapy in the treatment of lung cancer was performed by utilizing various research papers, academic published journals and relevant websites such as cancer.net, nhs.uk and cancerresearchuk.org. Multiple articles from different journals were used to collect the information for this literature review. The journals include Pubmed, Elsevier, Frontiers, ScienceDirect, Springer, etc. At the beginning, basic research was done about lung cancer immunotherapy and an initial list of research articles were established. Then an outline was prepared to conduct the review in a systematic way. The articles for the review were explored utilizing keywords such as lung cancer, immunotherapy, immunotherapy approaches, prospects, challenges, etc. After going through about 110 articles, 77 relevant papers from Scopus indexed journals were selected to gather information for the review.

Chapter 3

Cancer

3.1 What is Cancer?

Cancer, which is a malignant tumor, drives cells to divide abnormally and invade different parts of the body. Human body is made up with trillions of cells and cancer can develop in any place of the human body. Cell division is involved with multiplication and proliferation of cells. These newly generated cells are important for the human body and replace the old or damaged cells. This regular process can sometimes undergo disruption due to mutation and other chemical or environmental stimuli which may trigger abnormal growth and multiplication of cells. Tumors may or may not be malignant. Tumors which are cancerous can attack nearby tissues and also can spread to different parts of the body, which results in formation of new tumors called metastasis. Numerous malignancies result in solid tumors (sarcomas, carcinomas) but blood cancers including leukemia do not. Tumors which are not cancerous do not infect neighboring tissues. However, benign tumors rarely appear again after elimination, although cancerous tumors do appear again. However, benign tumors can be huge occasionally. Tumors such as benign brain tumors can generate harmful symptoms or even can be fatal (*What Is Cancer? - National Cancer Institute*, n.d.). In every country, cancer is a main reason for death, it is a great impediment to human life span. In 2019, World Health Organization (WHO) reported that cancer is the first or second main reason for death for the people who are below the age of 70 in 112 of 183 nations and also it places 3rd or 4th in another 23 countries (Sung et al., 2021).

3.1.1 How Does Cancer Develop?

Cancer is a hereditary disease caused by mutation in genes involved in how our cells work particularly how they divide and proliferate. DNA damage is induced by harmful compounds in the environment including chemicals in cigarette smoke; exposures to compounds which can cause toxic reactions such as benzene, asbestos, and nickel, cadmium, and vinyl chloride, benzidine, N-nitrosamines and ionizing radiation such as ultraviolet rays from sunlight as well as radiation from alpha, beta, gamma, all can cause genetic alterations that lead to cancer. Cancer can also be caused by Human papillomavirus, Epstein-Barr virus, hepatitis B virus and hepatitis

C virus, Kaposi's sarcoma-associated herpes virus, Merkel cell polyomavirus, and *Helicobacter pylori*, and also some bacteria. Damaged DNA cells are eliminated by the body before becoming malignant. Our bodies' ability to repair DNA decreases when we become older. This is the reason why people are more probable to develop cancer in later life. Different person's cancer is made up of a different group of genetic alterations. Further Alterations would happen as the cancer grows. Different cells can have multiple genetic alterations within the same locations (What Is Cancer? - National Cancer Institute, n.d.).

3.1.2 Signs and Symptoms of Cancer

Cancer is characterized by various symptoms. If symptoms do not improve after several weeks the person should seek medical help as soon as possible so that problems can be detected and addressed before it is too late. Illness, injury, benign tumors, and other disorders are responsible for the symptoms (Symptoms of Cancer - National Cancer Institute, n.d.).

The following are some of the symptoms that can be induced by cancer:

- Breast lumps, nipple discharge, or other changes
- Urine contains blood, painful urination
- Stools with blood
- Hoarseness or cough
- Nausea and vomiting
- Appetite changes
- Seizures and headaches.
- Throat irritation
- Swelling or lumps in the neck, underarm and stomach

3.2 Types of Cancer

Cancers are characterized based on histological type (tissue type from which cancer arises) and on the initial site of the body part where the cancer first appeared. Different forms of malignancies exist histologically such as carcinoma, sarcoma, myeloma, leukemia, and lymphoma.

The following are some of the most common cancers:

3.2.1 Carcinomas

Carcinomas are responsible for around 85% (85 of every 100 malignancies) and it is the most frequent type of cancer. Carcinomas begin in epithelial tissues and progress to other tissues. Squamous cell carcinoma, basal cell carcinoma, adenocarcinoma and also transitional cell carcinoma are some varieties of carcinomas that can be developed from various epithelial cells. Squamous cell cancer begins in the cells of the squamous epidermis. Squamous cells are flat cells which can cover surfaces and can be located in the skin, throat walls, and the food tube. Adenocarcinomas begin in adenomatous cells, which are glandular cells. Fluids are produced by glandular cells to keep tissues wet. Cells that may stretch as an organ expands are known as transitional cells and transitional epithelial tissues makes up, for example bladder lining. Transitional cell carcinomas are cancers that begin in these cells. Basal cell carcinomas begin from the innermost layer of skin which is lined by basal cells (*Types of Cancer | Cancer Research UK*, n.d.).

3.2.2 Sarcomas

Sarcomas begin in the body's connective tissues such as bones, cartilage, tendons, and also fibrous tissue and connective tissues help to support organs. Sarcomas are very common types of cancer but its prevalence rate is less than carcinomas. Bone sarcomas and soft tissue sarcomas are the two most common forms. Sarcomas are responsible for making up less than 1 percent of all cancers diagnosed each year. Bone sarcomas begin in the cells of the bone and most frequent types of soft tissue sarcomas begin in cartilage or muscle. Chondrosarcoma is a type of cartilage cancer. Rhabdomyosarcoma and leiomyosarcoma are cancers affecting muscle cells (*Types of Cancer | Cancer Research UK*, n.d.).

3.2.3 Leukemia

Leukemia can affect white blood cells. The bone marrow produces an excessive number of white blood cells. They do not function correctly because the blood cells are not fully developed. The blood becomes clogged with unusual cells and it is a rare disease. Leukemia is responsible for making up three out of every hundred cancer cases (3 percent). However, leukemia is the most prevalent cancer among children (*Types of Cancer | Cancer Research UK*, n.d.).

3.2.4 Lymphoma

Lymphomas and myeloma, malignancies of the lymphatic system, are two more forms of cancer. The lymphatic system means a collection of tubes as well as glands which is involved in purifying the body's fluid and combat infection. Lymphoma begins in the lymph nodes or cells in the lymphatic system. The reason behind developing lymphoma everywhere is because the lymphatic system can travel all over the body. It occurs when some lymphocytes (white blood cells) in the lymphatic system begin to multiply abnormally and they also don't end up the way they should. Before being fully matured, these cells begin to multiply and therefore they are unable to combat infection. The aberrant white blood cells begin to gather in lymph glands as well as other locations including the bone marrow and spleen and tumors can form as a result of this (*Types of Cancer | Cancer Research UK*, n.d.).

3.2.5 Multiple Myeloma

Multiple myeloma is a bone marrow disease that begins within plasma cells. The bone marrow generates plasma cells, which are a kind of white blood cell. Antibodies, also known as immunoglobulins, are produced by them to assist in the battle against infection. Plasma cells might develop abnormalities and grow uncontrollably. They produce antibodies that does not work well in the battle against infection **(***Types of Cancer | Cancer Research UK*, n.d.). It is termed multiple myeloma because the malignancy frequently infects various parts of the body including the skull, spine, pelvis, and also ribs (Multiple Myeloma - NHS, n.d.).

3.2.6 Melanoma

Melanoma cancer is a type of malignancy which begins in the melanocytes. It might infect just the skin or grow in the organs and bones. It is less prevalent than other types of skin cancer although it is more dangerous and invasive. Melanoma is a cancer that can be malignant or benign, malignant melanoma is deadly but benign melanoma is not deadly. It is important to recognize malignant melanoma skin cancer at an early stage in order to lower the fatality rate (Alasadi & M.ALsafy, 2015).

3.2.7 Germ Cell Tumors

Germ cell tumors, are very cancerous tumors which arise out of germ cells. These are the cells that evolve into sperm and eggs in the body. Germ cell tumors most commonly grow in the ovary or testicle because most germ cells are found there. However, while the embryo develops in the womb, germ cells can often be remained in other parts of the body. As a result, malignant tumors can form anywhere in the body where germ cells exist. Cancers arising from germ cells in other regions of the body are extremely uncommon. Extragonadal germ cell tumor (EGGCT) is the medical term for germ cell tumors that form outside of the ovaries or testicles. Germ cell tumors can begin in the brain, behind the abdomen which is called retroperitoneal cancer and the mediastinum which is a part of the chest (mediastinal germ cell tumors) (Germ Cell Tumours | Cancer Research UK, n.d.).

3.2.8 Neuroendocrine Tumor

A neuroendocrine tumor is an uncommon tumor that can grow in a variety of organs throughout the body. It has an effect on the cells that secrete hormones into the bloodstream (neuroendocrine cells). The symptoms of a neuroendocrine tumor vary based on where it is located in the body and what hormones it generates. A tumor in the digestive system, for example (gastrointestinal neuroendocrine tumor), may lead to diarrhoea, constipation, or stomach pains. Wheezing may be occurred by a tumor in lung (pulmonary neuroendocrine tumor) (Neuroendocrine Tumours - NHS, n.d.).

3.2.9 Central Nervous System Cancers

Central nervous system (CNS) cancers, that start in the tissues of brain and spinal cord which is called brain, spinal cord cancers such as gliomas, primary CNS lymphomas, meningiomas, pituitary adenomas as well as primitive neuroectodermal tumors (Types of Cancer | Cancer Research UK, n.d.).

3.3 Prevalence of Cancer

In 2020, there had been 19.3 million new cases of cancer with 10 million cancer deaths. Approximately 2.3 million new cases of cancer, female breast cancer has surpassed lung cancer, succeeded by lung cancer (11.4 percent), colorectal cancer (10.0 percent), prostate cancer (7.3 percent) and also stomach cancer (5.6 percent). However, with an approximate 1.8 million deaths, lung cancer still holds the leading position in cancer death, succeeded by colorectal cancer (9.4 percent), liver cancer (8.3 percent), and stomach cancer (7.7 percent), and also female breast cancer (6.9 percent) (Sung et al., 2021). In the twentieth century, cancer deaths rose by a larger percentage but it has steadily declined from its maximum in 1991 to 2018 which shows a total decline of 31%. This is due to smoking cessation and advancements in early diagnosis and treatment (Siegel et al., 2021). For both male and female, the total occurrence of cancer was higher in transitioned countries when it is compared to transitioning countries. Female breast and cervical cancer death rates were larger in transitioning countries vs transitioned countries. In 2040, the burden of global cancer is anticipated to be approximately 28.4 million which is a 47 percent increase from 2020 along with transitioning (64 percent to 95 percent) countries experiencing a greater increase than transitioned (32 percent to 56 percent) countries (Sung et al., 2021). Surprisingly, cancer rates are usually largest in areas with the greatest lifespan, level of education, and quality of life (Cancer Statistics - National Cancer Institute, n.d.).

Chapter 4

Immunosurveillance of Cancer

4.1 Innate Immunity

Innate immunity which includes macrophages and neutrophils is a generalized first line of defense with immediate response. Innate immune systems are stimulated by pathogens, smoking, and other environmental factors resulting in the release of cytokines. These cytokines have the potential to cause oncogenesis by promoting tumor spread, angiogenesis, and metastases, also they can stimulate cell damage (Domingues et al., 2014). Innate immune cells have the potential to induce cancer by direct interaction with tumor cells, increasing the activities of many other cells within the tumor microenvironment (Marcus et al., 2014). Individual's own immune system is a very important defense process that recognizes as well as destroys microorganisms such as bacteria, viruses, and other foreign substances through the integrated and combined stimulation of innate immunity and also adaptive immunity. The innate immune system plays an important role in identifying and removing tumor cells and this sort of immune response is facilitated by the rapid gathering as well as activation of macrophages, and dendritic cells (DCs), and natural killer (NK) cells, which can invade microorganisms and also tumors through endocytosis/cytolysis with the help of cytotoxic molecules/cytokines in a non-antigen specific manner (Canning et al., 2019). Innate cells, especially NK cells, demonstrate a collection of germline-encoded receptors that bind tumor-specific ligands aimed at providing tumor suppression (Sun, 2017). Natural killer (NK) cells had been initially presented as innate immune effector lymphocytes and provide fundamental cytolytic functions but further realistic overview of NK cells has advanced recently. NK cells can demonstrate a collection of stimulating inhibitory receptors, which are fine-tuned in order to build up self-tolerance, allowing success against viral infection and cancer growth (Vivier et al., 2011).

Tumor cell elimination can also be induced by NK cells via death receptor-mediated pathways including TRAIL and FasL. In a two-photon microscopy study, it has been found that infiltrating NK cells tumors form concise cytotoxic interactions accompanied by target cells that enables Natural Killer cells in order to destroy a huge number of infected or cancer cells quickly. Cytokines such as IFN-, TNF-, also growth factors including G-CSF, GM-CSF, a variety of

chemokines can be produced by activated NK cells and gamma/delta T cells (T cells). Particularly, IFN-γ have potent anti-tumor properties which includes promoting MHC I expression and making tumor cells more susceptible to CD8+ T-cell killing. In subcutaneous tumor transplantation models, gamma/delta T-cells are recognized as an important source of IFN-, and NK-cell-derived IFN- is correlated with patient survival in some cancers. TNF-α can cause cytotoxicity directly by inducing caspase-8-mediated apoptosis. Furthermore, tumor cells can enter a state of senility by combining IFN-γ and TNF-α. Therefore, cytokines released from innate cells can cause immediate anti-tumor impacts (Marcus et al., 2014). Based on the type of the antigenic drawback, the immunity of host T and B cells can be affected by natural killer cells either positively or negatively. The innovative idea, is that natural killer cells are more than just cytolytic effector cells against tumor cells. NK cell-mediated cytotoxicity as well as the production of cytokines have an effect on dendritic cells, macrophages, neutrophils, also provide NK cells along with regulatory activity that impact next T and B cell responses which is antigen specific (Vivier et al., 2011).

4.2 Adaptive Immunity

Adaptive immune system produces an antigen-specific immunity. Adaptive immunity is classified into two types: humoral immunity and cell-mediated immunity. B cells can stimulate humoral immunity and T cells can stimulate cell-mediated immunity. In the adaptive immunity system, at first the antigen is acknowledged by lymphocyte receptors, also immune cell clones are generated in order to invade that fixed antigen **(**Lakna, 2017). Many of the immune therapeutics currently available are based on the adaptive arm of the immune system. B cells, CD8+ cytotoxic T lymphocytes, CD4+ T helper lymphocytes all contribute to the coordination of an initial and long lasting anti-tumor response which destroys developing wounds. The method of immune editing has three phases, elimination, which is the immune system's tumor-killing response; equilibrium, which is the time for selection for the phenotypes that are non-immunogenic; 3rd and last phase is escape, in this phase, resistant cells can rapidly proliferate to create a cancer (Wang et al., 2020). Tumor-associated antigens (TAAs) are one of the most important immune response stimuli. They are significant because they activate the response of T cells, which provides a very necessary line of defense against tumor growth through a major histocompatibility complex (MHC). TAAs are produced by various mechanisms and introduced

to T cells through MHC class I molecule or II molecule on antigen presenting cells (APCs). This can cause T cells to become activated, resulting in the manifestation of molecules that are costimulatory, also the production of cytokines as well as chemokines. The result is to operate clonal growth of T cells and also the recruitment of other immune effector cells which includes elements of the nonspecific immune system (Spurrell & Lockley, 2014). Then T cells start to multiply and distinguish into armed effector cells when they come into contact with an antigen. Cells that are infected will be killed by cytotoxic T cells by causing apoptosis (Lakna, 2017). T helper cells or CD4+ T cells produce cytokines as well as chemokines which control various characteristics of the immune system. TH1 CD4+ T cells can promote immunity that is cell mediated through activating CD8+ T cells, also TH2 CD4 T cells can interact along with B cells promoting antibody mediated immunity. Cytotoxic CD8+ T cells are triggered by presentation of antigen through activation of MHC class I molecule or stimulation of CD4+ T cell. Finally, cancer cells are damaged through both straight cell-mediated cytotoxicity and also indirect antibody complement-mediated cytotoxicity (Spurrell & Lockley, 2014).

Chapter 5

Cancer Progression from Immune Escape of Tumor Cells

5.1 Tumor Microenvironment

In the early 2000, Robert Schreiber, a renowned immunologist enhanced and fine-tuned the cancer immunosurveillance supposition to emphasize the strong and reciprocal connections between cancer cells and the immune system. It is a three phase process which is called tumor elimination, Equilibrium, and Escape, explaining continuous war in the middle of the immune system and the cancer which assesses survival or growth of tumor as well as redesigns the immune landscape of the tumor microenvironment. The immunosurveillance concept is now widely recognized for relaying an essential metabolic method at the time of carcinogenesis as well as tumor progression, it also presents an incredibly valuable understanding of prospective immunotherapy intervention targets. Tumor immunosurveillance has three outcomes - (a) firstly Tumors are eradicated from the effective antitumor immunity of stimulated T cells as well as innate immune cells in the tumor microenvironment (TME), (b) Triggered immune cells coexistence which destroy some cancer cells as well as unresponsive immune cells, which results residual cancer survival as well as a state of tumor dormancy, and (c) Tumors and pro-inflammatory myeloid-derived suppressor cells can stimulate resilience of T cell, stop tumors from acknowledging by the host immune system in the immunosuppressive tumor microenvironment helping to promote tumor progression (Canning et al., 2019).

A developing tumor's tissue microenvironment is made up of spreading tumor cells such as the blood vessels, tumor stroma, infiltrating inflammatory cells, and also different tissue cells. It is a distinct environment that arises at the time of tumor progression and acts as a consequence to the tumor's interactions along with the host. TME helps to orchestrate molecular and cellular incidents in nearby tissues (Whiteside, 2008). Genetic transformation in tumor cells, also the rearrangement of tumor microenvironment components via reciprocal and dynamic crosstalk, can influence tumor formation and progression (Baghban et al., 2020). The TME is made up of B cells, T Cells, myeloid-derived suppressor cells, dendritic cells, tumor-associated macrophages, also a complex matrix of fibroblasts, lymphatics, blood vessels as well as cancer cells. The immune cells comprising tumor microenvironment has been illustrated in Figure 1. These TME

components are encircled by the extracellular matrix, which is a meshwork, made up of collagen and elastin fibers. The tumor microenvironment is made from a complex as well as the network of chemokines, cytokines, inflammatory factors, growth factors, and also matrix remodeling enzymes. The TME has a necessary role in immune escape, also in cancer progression (Osipov et al., 2019). The elements of the tumor microenvironment can exhibit a great influence on tumor development and progression. Tumor-associated fibroblasts are important to form and remodel the extracellular matrix, as well as serving as a source of growth factor that assists the growth of carcinoma cells. As the tumor grows larger, the formation of new blood vessels is crucial for tumor progression, while existing blood and lymphatic vessels may serve as paths for local invasion and distant metastasis. Other host cell lineages, such as mesenchymal stem cells, are capable of not only forming new carcinoma cells but also differentiating into the different cell types necessary to operate angiogenesis during cancer progression. Chemokines and cytokines, which are immune components of the tumor microenvironment can change the naive balance of pro regulatory as well as antitumor immune responses (Chew et al., 2012). Tumors grow and spread in complicated and evolving microenvironments, which affect their growth, metastasis as well as invasion. Cancer cells, and their surrounding microenvironments communicate frequently in this environment. Cancer cells, can interact along with their microenvironment in a dynamic way, which involves cell-cell/cell-free communication as well as the mediators which allow these communication. Mediators are basically molecules/factors/vesicles that can transfer a genetic information in the middle of interacting cellular or non-cellular cells (Baghban et al., 2020).

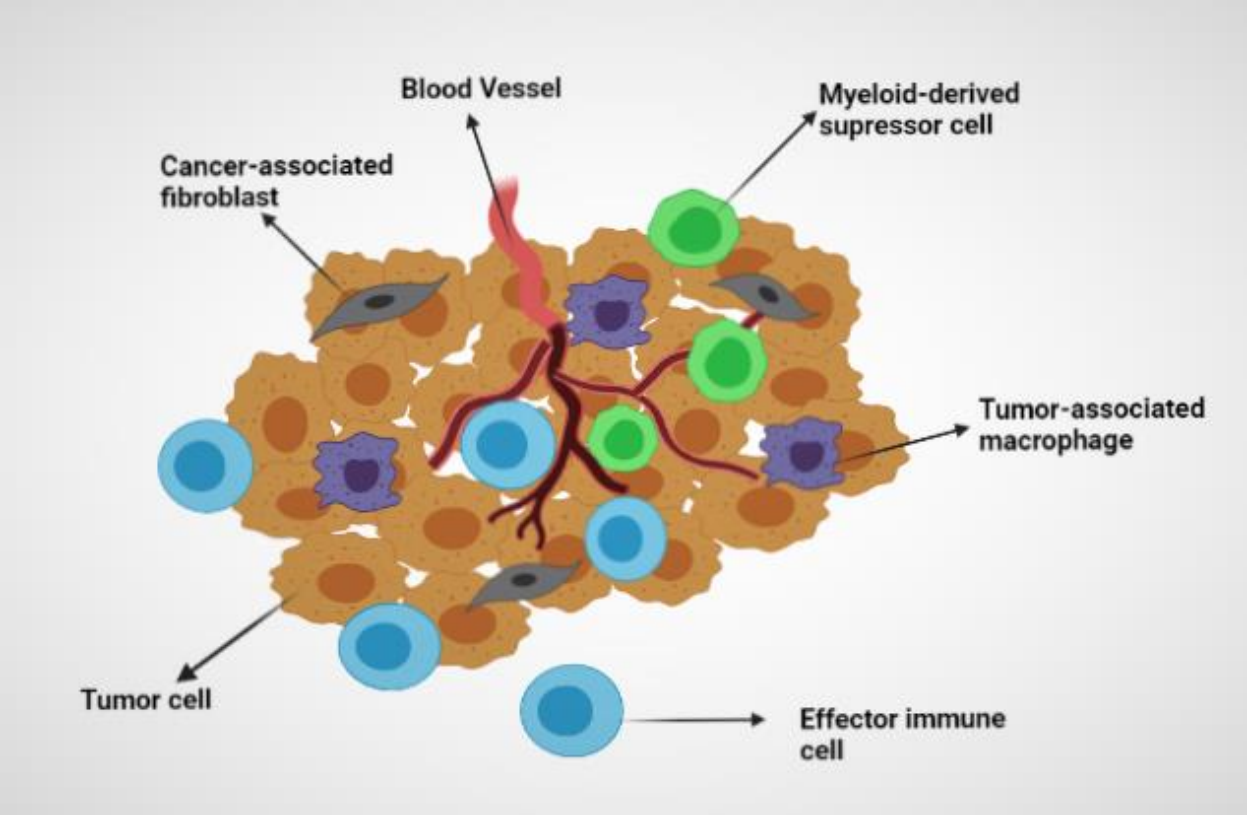

Figure 1: Tumor microenvironment. Tumor microenvironment consists of various types of cells including myeloid-derived suppressor cell, T lymphocytes, B lymphocytes, cancer-associated fibroblasts, natural killer cells, dendritic cells, tumor-associated macrophage, neutrophils, basophils, blood vessels, tumor cells, mast cells as well as endothelial cells (Adapted from Yang et al.,2016).

5.2 Immune Checkpoints

Immune checkpoints are inhibitory paths that are essential to maintain self-tolerance. Tumors utilize specific checkpoint paths to avoid immune surveillance. In tumor microenvironment, inhibitory ligands and receptors controlling the effector functions of T-cell, are frequently overexpressed. Immune checkpoint blockade liberates the immune system, also resulting in T-cell responses that are antigen specific. Inhibitory receptors such as CTLA-4, PD-1 are the most discussed checkpoint receptors for immune responses (Anagnostou & Brahmer, 2015). Immune checkpoints are activated to stop the occurrence of autoimmunity. CTLA-4 can be expressed by

activating CD8+ that binds to ligands B7 along with large affinity and restricts more activation of T-cell by CD28 (Domingues et al., 2014). CTLA-4 initially controls CD4+ T cells, and also increases the regulatory T cells immunosuppressive activity. During an inflammatory response, PD-1 can mediate immune resistance inside the tumor microenvironment ells effector through suppressing the activity of T cell effector in peripheral tissues. PD-1 inhibitor is found on tumor-infiltrating lymphocytes which can recognize tumor cells, principally CD4+ T cells, B cells, monocytes, natural killer cells, and also dendritic cells. PD-1 obstructs kinases which involve T-cell activation and it is abundant on regulatory T cells, which may increase their proliferation (Anagnostou & Brahmer, 2015). PD1 can act as a T-cell surface receptor which hinders the immune reaction and has an ability to bind to PD-L1 in the APC or tumor cell. PD1 functions by restricting activity of T cells within the tumor microenvironment whereas CTLA-4 antibodies act by restricting T-cell activation introduction in the lymph nodes (Domingues et al., 2014). Innate and adaptive immune resistance are the two mechanisms in which immune checkpoint blockade are expressed on cancer cells, via oncogenic signaling in the tumor microenvironment which is called innate immune resistance. Adaptive immune resistance is called when induced response to inflammatory signal generated by an active antitumor immune reaction. Inhibiting the CTLA-4 and PD-1 paths can be seen in several preclinical studies to improve intratumoral immune responses, and blocking immune checkpoints. As a result of cancer cells expressing various inhibitory ligands and tumor infiltrating lymphocytes expressing various receptors and antitumor immune reactions may be increased by multi layered immune checkpoint blockade (Anagnostou & Brahmer, 2015).

Chapter 6

Lung Cancer

6.1 Introduction to Lung Cancer

Lung cancer is an aggressive cancer as well as spreads rapidly. Lung cancer is the major reason for mortality in the USA. It kills more people in the United States (Hassan Lemjabbar-Alaouia et al., 2017) and increase the rates of mortality from cancer in men while it ranks third in occurrence and second in death rates in women after breast and colorectal cancer. Men have roughly twice the incidence and mortality rates as women, though the male-to-female proportion differs greatly throughout regions. It is commonly diagnosed in men within 36 countries, and a major reason for death from cancer within 93 nations. In 25 countries within Northern America, and Oceania as well as parts of Europe, lung cancer stands as a major cause of mortality among women with the greatest prevalence rate in the nations such as Northern America, as well as Northern, Western Europe, Micronesia/Polynesia, and also Australia, New Zealand, Hungary (Sung et al., 2021). The progression from a normal cell to malignant lung tumor cell can occur in stages along with a huge series of genetic as well as epigenetic changes. This changes can cause invasive cancer by clonal expansion. Supporting the progress of cancer from the initial stage, the continued increase in genetic, epigenetic defects obtained at the time of clonal expansion affects invasion mechanism, metastasis, as well as resistance of cancer therapy. However, the identification of these molecular changes is very crucial for treatment and disease prevention and early detection. Knowledge about the tumor characteristics of patients as well as other genetic information can help to select the treatment (Hassan Lemjabbar-Alaouia et al., 2017). Cigarette smoking is the main predisposing factor for lung cancer including other risks such as environmental tobacco smoke, and occupational exposure to asbestos and radon progenies, and also diet are. Environmental tobacco smoke can increase the risk along with a dose-dependent correlation between the relative risk and degree of exposure. Early detection and treatment of disease and chemoprevention and smoking avoidance as well as cessation can all help to reduce the occurrence of lung cancer and thus its associated mortality. Prevention of smoking and also cessation campaigns designed to lower smoking rates and thus can decrease lung cancer risks. However, lung cancer cases keep rising, with the massive growth in women being. Our concern

in the future should be to mitigate the influence of the recognized external sources of risk as well as expanding knowledge about genetic and molecular changes base on carcinogenesis (Dela Cruz et al., 2011).

6.2 Types of Lung Cancer

Lung cancer has two subtypes: small-cell lung carcinoma (SCLC), non-small-cell lung carcinoma (NSCLC), which reports 15% and 85% of all lung cancers (Zappa & Mousa, 2016).

6.2.1 Small Cell Lung Carcinoma (SCLC)

Small cell lung cancer (SCLC) is a deadly cancer and strongly linked to cigarette smoking. Patients often present with short-term symptoms and often 60-65 percent of cases with metastatic disease (Bernhardt & Jalal, 2016). Small cell lung carcinomas (SCLC), are aggressive, which arise from the hormonal cells in lungs and account for 10%–15% of all lung cancers and also quickly spreads into submucosal lymphatic vessels, regional lymph nodes. (Hassan Lemjabbar-Alaouia et al., 2017).

6.2.2 Non-Small Cell Lung Carcinoma (NSCLC)

NSCLC, a major reason for cancer-related death in the United States and is the most avoidable type of cancer mortality (Araujo et al., 2020). NSCLC can cause the majority of cases among all the lung cancer types. Cigarette smoking is considered to be the main risk factor for NSCLC including some environmental factors and also genetic risk factors (Zappa & Mousa, 2016). Non-small cell lung cancer (NSCLC) remains within a group that include squamous cell carcinoma and adenocarcinoma as well as large cell carcinoma (Non Small Cell Lung Cancer - PubMed, n.d.). Other lung cancer subtypes that are included in NSCLC: adenosquamous and carcinoma, and sarcomatoid carcinoma, and also non-small cell neuroendocrine tumors.

i. Squamous Cell Carcinoma

Squamous cell lung cancers, known as epidermoid carcinoma which account for approximately 25% to 30% of lung cancer cases and typically develop within the main bronchi (Hassan Lemjabbar-Alaouia et al., 2017). It typically begins from the tracheobronchial tree's origin, however, more cases are now being diagnosed on the lung's periphery (Non Small Cell Lung

Cancer - PubMed, n.d.). It develops from the air passages of the lungs and this subtype has a strong link to smoking (Zappa & Mousa, 2016).

ii. Adenocarcinoma

Adenocarcinoma accounts for hardly 40% of all lung cancer cases and develops from alveolar cells, type II, that are small airway epithelial cells that can secrete mucus as well as other substances. It is the most usual form in both smokers, nonsmokers of all ages. It is more common on the lung's periphery and the reason for this can be, adding more filters in cigarettes that prevent big molecules from reaching the lungs. This can lead to peripheral injuries by causing deep inhalation of cigarette smoke. Adenocarcinoma can grow more slowly and possess a greater possibility of being detected before it spreads throughout the lungs (Zappa & Mousa, 2016).

iii. Large Cell Carcinoma

5–10% of lung cancers are large cell (undifferentiated) carcinomas. There is no proof of glandular maturation in this type of carcinoma, and so it is commonly detected by exclusion of other possibilities. Large cell carcinoma, a type of non-small cell lung cancer, frequently starts in the main part of the lungs, spreading to adjacent lymph nodes as well as the chest wall and also distant organs. Smoking is strongly linked to large cell carcinoma tumors (Zappa & Mousa, 2016).

iv. Adenosquamous Carcinoma

Adenosquamous lung carcinoma contains elements of lung adenocarcinoma (ADC) as well as lung squamous cell carcinoma (SCC). It is a very uncommon subtype of lung cancer, accounting for 0.4 to 4% of all lung cancers. ASC has three subtypes based on the part of the two components: ADC-predominant ASC and SCC-predominant ASC and also structure-balanced ASC. ADC can develop from type II alveolar epithelial cells, whereas SCC can develop from basal cells within the proximal bronchus. It is still not clear whether ASC is a mash-up of ADC and SCC (C. Li & Lu, 2018).

v. Sarcomatoid Carcinoma

Pulmonary sarcomatoid carcinoma (PSC) is an uncommon subtype of NSCLC which reports for 0.1to 0.4% of all lung carcinomas. In the early stage, PSC possesses a poor prognosis than other types of NSCLC. PSC is more common in people who are older and smoke heavily and also possess an upper lobe predisposition (Karim et al., 2018).

vi. Large Cell Neuroendocrine Carcinoma

Large cell neuroendocrine carcinoma (LCNEC) is a very invasive, unusual neoplasm that is difficult to diagnose and treat. It is responsible for approximately 1.6–3.1 percent of all lung cancers. WHO now classifies LCNEC as a histologically high-grade non-small cell cancer, classifying it as a form of large cell carcinoma. LCNEC has a remote metastasis rate of 65% and a poor prognosis, even in the early stages and survival rates comparable to small-cell lung carcinomas (SCCs) (Yuan et al., 2014).

6.2.3 Pleural Mesothelioma Cancer

Pleural mesothelioma specifically targets the lung lining. Pleural mesothelioma symptoms include chest pain, shortness of breath, fatigue, loss of appetite, high temperature, fever, and sweating. Mesothelioma is often induced by asbestos exposure which is a group of minerals composed of microscopic fibers that was once widely used in construction. These small fibers can quickly enter the lungs and become trapped causing long-term damage to the lungs (Mesothelioma - NHS, n.d.).

6.3 Treatment Options for Lung Cancer

6.3.1 Treatment for Small Cell Lung Carcinoma

The treatments for the patients with SCLC, are destined based on the study of histology and stage as well as the patient's general health condition and also chronicity. Recommended treatment choices for limited-stage small-cell lung cancer are: platinum-based chemotherapy, radiation therapy and prophylactic cranial irradiation and only combination chemotherapy as well as surgery which is followed by chemotherapy/chemoradiation therapy (Joshi et al., 2013). Chemotherapy and palliative therapy are the treatment options for repeated SCLC. Despite the

fact that conventional chemotherapy and also conventional radiotherapy can produce significant initial responses in case of SCLC but disease repetition is very prevalent. SCLC, which is not treated, can be the greatest assertive type along with an average survival of 2-4 months after diagnosis. Even though surgery or chemotherapy itself can enhance patient survival with LS-SCLC, combination therapy is shown to enhance long-term survival even more. However, combining traditional chemotherapy along with thoracic radiation therapy (TRT), in particular, raises overall survival when being compared to single chemotherapy treatment. In Extensive Stage Small cell lung cancer, bevacizumab was used to inhibit circulating vascular endothelial growth factor (VEGF) (Hassan Lemjabbar-Alaouia et al., 2017). Bendamustine, which is well-tolerated and successfully used even as a single agent in second-line settings as well as third-line settings in patients who have relapsed or refractory SCLC (Lovly et al., 2012).Several research has examined cancer immunotherapy as a prominent treatment option for SCLC. The BEC2 vaccine or BCG vaccine merges a mAb which copies the glycosphingolipid GD3. This glycosphingolipid GD3 is conveyed in SCLC along with an adjuvant, bacillus Calmette-Guerin. The BEC2 vaccine or BCG vaccine can induce antibody production against b-series ganglioside GD3 in patients with melanoma. A new vaccine (INGN-225) designed to target the p53 protein is being approved for treatment of SCLC (Hall et al., 2013). The epidermal growth factor receptor (EGFR) alteration is less frequent within SCLC than NSCLC and just 4% carry the alteration. Patients who are chemosensitive or have chemo-refractory relapsed SCLC were given gefitinib in a phase II clinical trial (Hassan Lemjabbar-Alaouia et al., 2017).

6.3.2 Treatment for Non-Small Cell Lung Carcinoma

Lung cancer therapy strategies include traditional surgery, radiation therapy, conventional chemotherapy as well as targeted therapy. Surgery can be done as a primary therapy for resectable, also operable in early Stage I and stage II cancer. It offers the best chance of prolonged survival. Life expectancy of five year after removing a tumor with surgery is 60% to 80% for NSCLC stage I patients and also 30% to 50% for NSCLC stage II patients (Hassan Lemjabbar-Alaouia et al., 2017). Primary radiotherapy, for example stereotactic body radiotherapy (SBRT) for unresectable tumors, can be used for patients, who deny surgical resection or possess unresectable tumors. Post-surgery radiotherapy, on the other hand, is not advised for patients in stages I and II. Platinum-based chemotherapy can be advantageous for

NSCLC stage II patients, also is the suggested treatment strategy for the ones who have been completely resected (Howington et al., 2013). NSCLC stage III differs from removable tumors along with lymph nodes metastases to uneradicable disease with numerous nodal sites. Treatment strategies for stage III NSCLC patients, such as traditional radiotherapy, conventional chemotherapy as well as resection with surgery are ascertained by the area of the tumor. For patients who have resectable stage IIIA NSCLC, the standard treatment is surgery followed by chemotherapy. Standard treatment for uneradicable stage IIIA patients could be a sequential or contemporaneous chemoradiation therapy as well as external radiation therapy for the ones who are not allowed to treat with combination therapy. The various treatments for stage IIIB NSCLC are destined by the tumor location as well as the patient's performance status. These patients require the standard treatment consisting of a sequential combined chemotherapy, or external radiation therapy (Hassan Lemjabbar-Alaouia et al., 2017). Patients who have advanced stage IIIB and stage IV non squamous carcinoma, the function of targeted therapies particularly bevacizumab, an anti-vascular endothelial growth factor agent, has been investigated. When combined with chemotherapy, bevacizumab enhanced survival compared to chemotherapy alone (Collins et al., 2007). However, palliative care treatment for NSCLC stage IIIB can consist of only external radiation therapy to ease pain as well as other symptoms and also enhance the status of life. Forty percent of patients who are newly diagnosed with NSCLC have stage IV, the most advanced stage of NSCLC. In palliative care, external radiation therapy, combined chemotherapy and immunotherapy, and also targeted therapy as well as other laser therapy or internal endoscopic radiation therapy can be needed, these are standard treatments available for stage IV NSCLC disease. Surgery, like radiation therapy, may be applied for some cases to ameliorate disease-related signs (Hassan Lemjabbar-Alaouia et al., 2017). If the cancer has not spread too much but surgery is not an option, in that case patients may decide to offer radiotherapy to kill the cancerous cells. This may be mixed with chemotherapy in some cases which is known as chemoradiotherapy. Chemotherapy and/or immunotherapy are generally recommended if the cancer has spread too much for surgery or radiotherapy to be efficacious. If the cancer returns after you have completed chemotherapy, another course of treatment may be suggested. If the cancer possesses particular alteration, then targeted therapy can be suggested in lieu of chemotherapy treatment or in addition to conventional chemotherapy (Lung Cancer - Treatment - NHS, n.d.).

6.3.3 Treatment for Pleural Mesothelioma

Mesothelioma treatment options such as traditional surgery, conventional chemotherapy, and radiation therapy are hardly remedial. Clinical study of single-modality therapy along with pleurectomy and chemotherapy, also radiotherapy did not demonstrate greater improvements in survival period than supportive care (Hassan Lemjabbar-Alaouia et al., 2017). Pemetrexed with cisplatin or carboplatin is the most commonly used chemotherapy drug for treating pleural mesothelioma. Chemotherapy for pleural mesothelioma intends to improve quality of life by extending life and shrinking the cancer. Radiation therapy, also known as radiotherapy, the use of x-rays in order to destroy tumor cells may be used in different phases and in various ways in pleural mesothelioma. Extrapleural pneumonectomy (EPP) eliminates the tumor and the pleura, the invaded lung, and the diaphragm as well as a pleurectomy along with pulmonary decortication eliminates the pleura and also the disease as much as possible (Mesothelioma | Causes, Symptoms & Treatments | Cancer Council, n.d.). Recent research has revealed that cancers with activating alterations in some proto-oncogenes can be specifically susceptible to targeted treatments aimed directly against the protein mutation. Mesothelin, WT1 analogue peptide vaccine, dendritic cell vaccine, and other immunotherapy approaches, have been evaluated in patients with mesothelioma (Hassan Lemjabbar-Alaouia et al., 2017).

Chapter 7

Immunotherapy in Lung Cancer Management

7.1 Active Specific Immunotherapy

Active specific immunotherapy entails inducing a powerful, cell-mediated, cytotoxic immune response against tumor cells. If immunomodulatory agents can strengthen the immune system against cancer then active specific immunotherapy continues to serve as a navigation system (Hanna et al., 2014). Specific activation of the individual's own immune system offers significant possibility for attaining non-toxic and long-lasting antitumor responses (Winter et al., 2011). Cancer vaccines are contemplated as active immunotherapy because they enhance the immune system's capability to combat cancer. Active immunotherapy is classified into two types: prophylactic and therapeutic. Prophylactic cancer vaccines are intended to prevent cancer in patients who are predisposed to it due to a genetic instability or environmental factors. Moreover, such vaccines focus on infections that can lead to cancer development. Therapeutic vaccines are designed to treat a pre-existing tumor. A solid accessible tumor can be eliminated by surgery, chemotherapy, or radiation therapy after cancer has been detected. Following surgery, the patient is vaccinated in order to stimulate a specific immune response that kills residual cancer cells and thus prevents recurrence. Dendritic cell (DC)-based vaccines have grown in popularity as a relatively safe form of cancer vaccine therapy in humans (Kokate, 2017).

7.2 Active Non-Specific Immunotherapy

Although not antigen-specific, active non-specific immunotherapy, also known as immunomodulatory therapies, entails both the innate parts and adaptive parts of the immune system. These treatments include cytokines like interleukins and interferons, immune-stimulatory agents like CpG oligonucleotides and Bacillus Calmette Guérin, antibodies against receptors like CTLA-4, PD-L1 and PD1 inhibitors, and indoleamine-2,3-dioxygenase enzyme inhibitors. The downstream mechanisms can be classified as direct anti-tumor effects, immune suppression reversal, activation of innate immunity, and also non-specific antigen T-cell activation (Monjazeb et al., 2012).

Chapter 8

Immunotherapy Options for Lung Cancer

8.1 Cancer Vaccines

A vaccine is made up of tumor-associated antigens that has the ability to produce an immune response, which can be recombinant proteins, peptides, whole tumor cells, gangliosides and is combined along with an adjuvant all the time to boost the immune system. In case of lung cancer, various vaccination techniques have been tested. MAGE-A3, melanoma antigen 3, is a potential approach for cancer immunotherapy as it is solely demonstrated on the cellular surface of tumor cells. It is not elicited in normal tissue with the exception of germline cells in male that do not present the antigen due to an absence of MHC molecules. MAGE- A3's function is not known, but its presence has been linked to a poor prognosis of lung cancer. The manifestation of MAGE-A3 has been found in 35 percent of early-stage NSCLCs and also up to 55 percent of late-stage NSCLCs. The MAGE-A3 vaccine is made up of a genetically recombinant fusion protein (MAGE-A3 and *Haemophilus influenzae* protein D in mixture along with an immune-strengthening adjuvant) (Declerck & Vansteenkiste, 2014). 182 patients, in total who were in stage IB/II NSCLC were decided to enroll in a placebo-controlled, double blind, randomized phase II study to take either the MAGE-A3 vaccine (n = 122) or a placebo group (n = 60). The findings of this study revealed that no notable change was noticed in disease-free interval (DFI), disease-free survival (DFS), or overall survival (OS) between the placebo groups and vaccinated groups. Recurrence has been noticed in 35 percent of participants who administered the MAGE-A3 vaccine after 44 months, when compared to 43% of individuals in the placebo group. Amusingly, IgG antibodies directed against MAGE-A3, have been identified in all MAGE-A3 vaccinated individuals, indicating that this vaccine is capable of inducing a particular immune response. These findings from the study demonstrated efficacy of the MAGE-A3 vaccine while minimizing toxic reactions (Aldarouish & Wang, 2016).

Belagenpumatucel-L is a therapeutic and an allogeneic whole-cell vaccine obtained from four radioactive cell lines of NSCLC- two adenocarcinomas, one large cell and one squamous cell carcinoma, transfected with a plasmid containing the transforming growth factor-beta 2 (TGF-2) transgene which can regulate the TGF-2. In a Phase II trial, the safety as well as effectiveness of

belagenpumatucel-L were studied in 75 individuals with stage II-IV NSCLC (Massarelli et al., 2014). Patients were separated into groups and also given one of three doses in a randomized, dose-variable Phase II clinical trial. A partial response rate (15%) was observed in the sub-group of 61 late-stage (IIIB and IV) patients who are able to be assessed. Interestingly, patients who received high doses had a better overall survival (OS) than patients who received low doses with an approximate 2 years survival rate of 52% vs 20%. In a Phase III clinical trial of 532 patients with stage III or stage IV NSCLC who did not gain improvement after receiving platinum-based chemotherapy were separated into two groups where 270 patients received belagenpumatucel-L vaccine and other 262 received a placebo. This clinical trial did not achieve its goal of improving overall survival (Aldarouish & Wang, 2016).

CIMAvax is a vaccine based on human recombinant epidermal growth factor (EGF). Phase I or Phase II studies have ensured the vaccine's ability to boost immune response in combination with chemotherapy and adjuvant. NSCLC proliferation, apoptosis, angiogenesis, and metastasis are all influenced by signaling of epidermal growth factor receptors (EGFR). Strong expression of EGFR is common in lung cancer. EGFR gene mutations resulting in changed EGFR function are linked to a greater response rate to the tyrosine kinase inhibitors of the receptor's interior part (Declerck & Vansteenkiste, 2014). CIMAvax-EGF vaccine was invented by Cuban scientists for the treatment of NSCLC adult patients who are in stage IIIB/IV after first-line chemotherapy by boosting their immune response to create anti EGF antibodies as well as decreasing EGF in serum. After first-line chemotherapy, 80 NSCLC individuals were randomly allocated to take either the Bacillus Calmette Guérin or the EGF vaccine in a Phase II study and vaccinated patients (51.3%) had a clear anti EGF antibody response. Moreover, serum concentration of EGF was clearly reduced in 64.3 percent of patients who were vaccinated. Surprisingly, the scientists discovered a very strong correlation between rate of survival and elevation of antibody response as well as decrease in EGF serum. CIMAvax vaccine also had a good safety profile (Aldarouish & Wang, 2016).

TG4010 is a therapeutic cancer vaccine based on viral vector made up of attenuated Ankara virus that has been modified genetically to demonstrate MUC1 as well as interleukin-2 (IL-2). IL-2 is an immunoadjuvant due to its ability to reverse the T-cell response suppression mediated by the cancer-associated MUC1 (Massarelli et al., 2014). 148 patients who were in stage IIIB or stage

IV NSCLC were separated into two groups in a phase II study.74 patients were given TG4010 in combination with chemotherapy, cisplatin with gemcitabine, while the remaining 74 patients were given only chemotherapy. The findings showed that the 6-month progression-free survival in the TG4010 along with the chemotherapy group was 43.2 percent (32/74) and 35.1 percent (26/74) in the group who were given chemotherapy alone. Progression free survival in individuals with normal levels of activated natural killer cells, was clearly greater than the control group (58 percent vs 38 percent). Furthermore, patients who have a normal level of NK cells showed a longer median overall survival (OS) than patients who have high levels (18 months vs 11.3 months) (Aldarouish & Wang, 2016).

In a Phase II trial of 83 NSCLC individuals, the G-VAX vaccine, an autologous tumor cell vaccine transfected with an adenovirus and contains the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene as an adjuvant has shown very good results (Declerck & Vansteenkiste, 2014). However, in a Phase III study, G-VAX ended with failure on clinical efficacy tests. The failure could be responsible for the approach's insufficient immunogenicity and changes in vaccine preparation required for commercial scale-up (Farkona et al., 2016).

The BEC2 bacille Calmette–Guérin vaccine which is an anti idiotype antibody vaccine against the GD3 ganglioside has been tested in patient who suffer from small-cell lung cancer (SCLC) and it was combined along with bacille Calmette–Guérin as an adjuvant to enhance the host immune system. Gangliosides are expressed on the surface of tumor cells and are engaged in cell matrix adhesion, cell differentiation and cell-cell recognition. A Phase III study was conducted internationally in 515 patients who are in the limited-stage of SCLC after showing a huge response to chest radiotherapy and chemotherapy was started based on a small study with 15 patients. Although the toxicity was low, no effect was shown on OS, PFS, or life quality. Another novel anti-idiotype vaccine for NSCLC is racotumomab which is formerly known as 1E10. It is similar to neu-glycosylated gangliosides, neu-glycosylated sialic acid-containing ganglioside is an alternate to the normal neu-acetylated sialic acid ganglioside that has been found in transformed cells, produces neu-glycosylated sialic acid-containing ganglioside, a potentially essential therapeutic target immunotherapy (Declerck & Vansteenkiste, 2014).

DC-based vaccines perform by delivering activated autologous dendritic cells to the patient, generating an immune response that is specific to the neoplasia. A Phase III trial found that patients who are treated with adjuvant DC vaccine together with surgery had a lower recurrence rate than patients who are treated with surgery alone (10 percent vs. 25 percent). During the previously stated trial, a translational study was carried out to identify authentic biomarkers for effective DC vaccine therapy, including a decrease in macrophage inflammatory protein-1alpha, an increase in NK cell counts as well as a normal CD4+/CD8+ ratio (Yang et al., 2016). The mechanism of action of cancer vaccines is depicted in Figure 2.

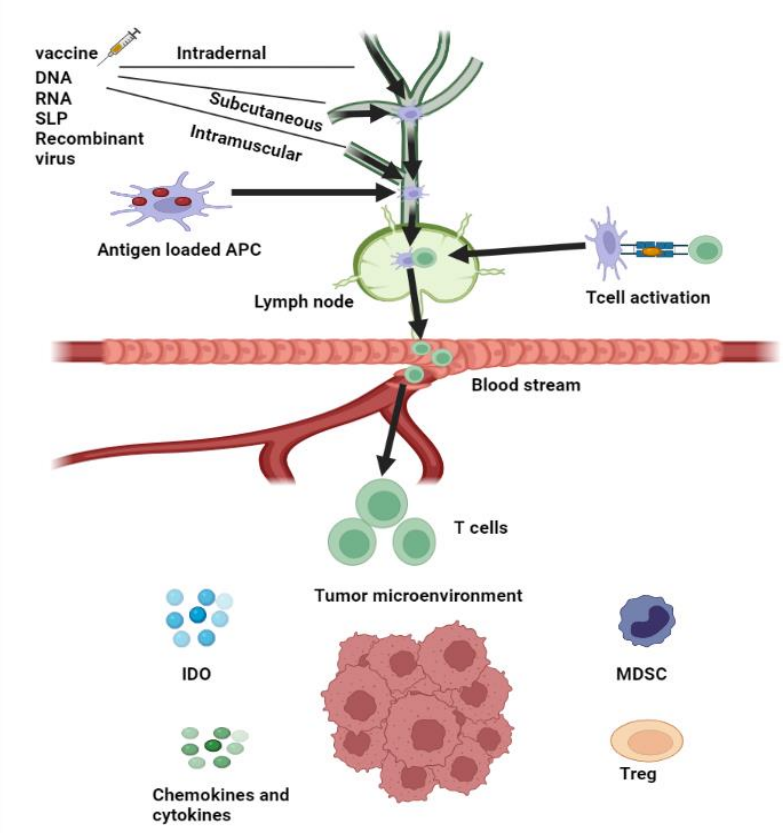

Figure 2: Mechanism of action of cancer vaccines. Antigen-bound dendritic cells (APCs) move via afferent lymph to the lymph nodes where T cell activation occur. The activated T cells travel via efferent lymph, thoracic duct, blood stream and reach the tumor cells. T cells which are vaccine induced clears the components of tumor microenvironment involved in impairing T cell migration, function and expansion such as Tregs, MDSCs, chemokines, cytokines, IDO (Adapted from Saxena et al., 2021).

8.2 Cytokines

The use of cytokines, particularly the one's secreted by Th1 cells, such as IL-2 was the first type of immunotherapy to be applied for NSCLC treatment (Yang et al., 2016). In the tumor microenvironment, tumor and stromal cells can secrete cytokines. Even though cytokines are essential factors in maintaining proper organ function, they can also play a role as tumor-promoting or tumor-suppressor molecules in the context of neoplasia (Marrugal et al., 2018). Cytokines are basically small proteins that are generated by different cells such as immunocytes and also non-immunocytes which perform as molecular messengers in order to contact successfully with other cells. Cytokines can play a variety of roles in the immunity cycle of tumor, including T cell priming and activation, antigen presentation, effector T cell infiltration at the cancer area, and also cancer cell death. The direction of naive CD4+ T cell differentiation is controlled by cytokine-mediated signaling pathways, which determines the impacts of anticancer immunity. TGF-β (transforming growth factor) signaling is required in CD4+ T cells for the distinction of regulatory T cells (Tregs) as well as T helper type 17 (Th17) cells. Furthermore, Th17 cell distinction as well as clonal expansion actually require a mixture of cytokines such as (IL-6, IL-21, IL-23, IL-1, and TGF-). Th17-secrete IL-17 which directs macrophages and neutrophils to cancer areas. Th17 cells exhibit antitumor activity in the melanoma microenvironment by enhancing the CD8+ T cell and Th1 cell activities. For differentiation and development, Th2 cells require IL-10, IL-11, IL-4, and IL-13 whereas Th1 cells require IL-12, IL-18, IL-1β and interferon (IFN)-γ. Th1 cells can stimulate secretion of IFN-γ and increase the cytotoxic activity of natural killer (NK) cell, in contrast, Th2 cells can suppress anticancer immune responses and block Th1 cell differentiation –γ release (Qiu et al., 2021). Nevertheless, human recombinant IL-2 administration did not show any clinical benefit in Phase II trial. In fact the therapy was not very well accepted resulting in cardiac and pulmonary toxicity of grade 3–4. However, a Phase II trial found that adding IL-2 to chemotherapy (gemcitabine+docetaxel) improved response rates along with good tolerability in patients accompanied by an advanced stage of NSCLC. In a Phase III randomized trial of IL-2 in combination with chemotherapy using a cisplatin doublet, these findings were not repeated. A subsequent study found that while IL-2 was used in combination along with the melatonin of pineal neurohormone, it resulted in a partial response of 20%, and a 50% disease which is long lasting in 20 advanced stage NSCLC patients (Yang et al., 2016).

8.3 Checkpoint Inhibitors

Immunotherapy uses immune checkpoint inhibitors which is a game changer for many cancers. The most valuable immune checkpoints are proteins found in T lymphocyte surfaces such as PD-1 molecule, which plays the role in regulating T cell function in peripheral tissues as well as the CTLA-4 molecule that regulates lymphocyte functions within lymph nodes during antigen presentation (Wojas-Krawczyk et al., 2019). The mechanism of action of immune checkpoint inhibitors is depicted in Figure 3.

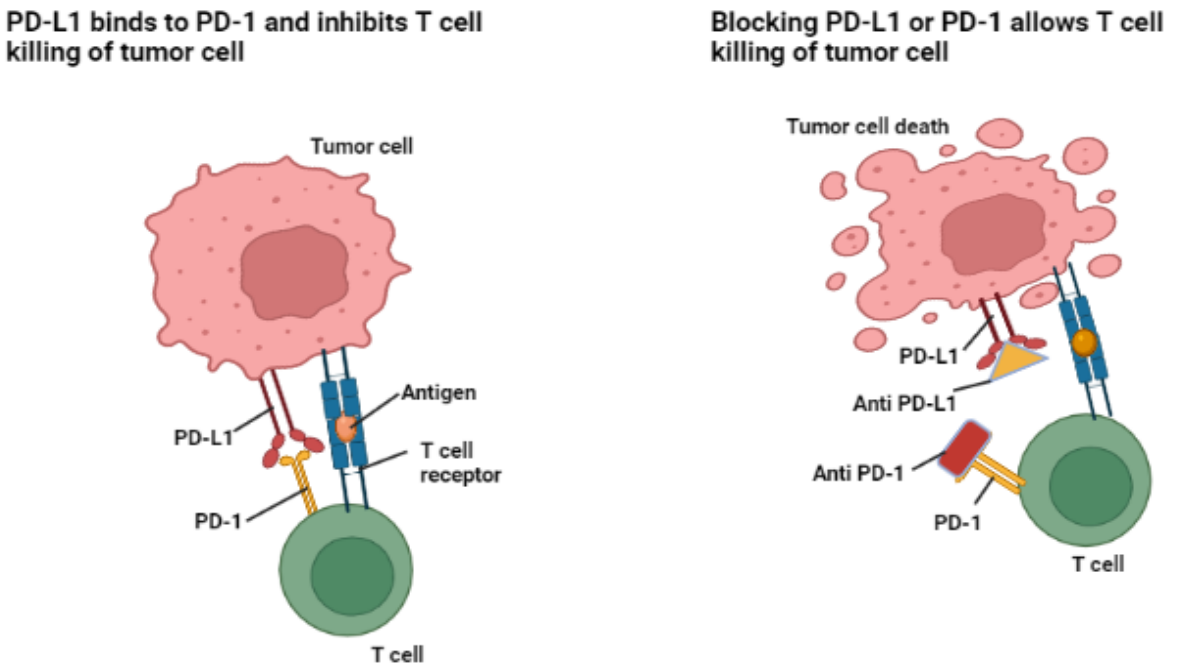

Figure 3: Mechanism of action of immune checkpoint inhibitors. Cancer cells express PD-L1 and T cells express PD-1 on its surface. PD-L1 binds to PD-1 and prevents T cells from destroying cancer cells. Administration of immune checkpoint inhibitors such as anti-PD-1 and anti-PD-L1 prevents the binding of PD-L1 to PD-1, permits the T cells to recognize and destroy cancer cells (Adapted from Immune Checkpoint Inhibitors - National Cancer Institute, n.d.).

8.3.1 Anti CTLA-4 Antibodies

CTLA-4 are receptors only expressed on T-cells. It can act as a negative costimulatory signal which inhibits activation and proliferation of T-cell to sustain self-tolerance and prevent autoimmmunity (Bertrand et al., 2015). In the year of 2011, the Food and Drug Administration (FDA) gave approval to Ipilimumab, anti-CTLA-4 monoclonal antibody with a dosage of 3 mg/kg in every 3 weeks for four cycles in patients with advanced melanoma. In a phase II clinical trial by using paclitaxel and carboplatin (with Ipilimumab or without Ipilimumab) in stage IV NSCLC, Ipilimumab showed improvement in immune-related progression-free survival (irPFS), when it was given after chemotherapy. The rationale for administering Ipilimumab after chemotherapy was to allow the release of antigen before immune modulation starts with Ipilimumab (Anagnostou & Brahmer, 2015).

8.3.2 Anti PD-1 and Anti PD-L1

NK cells, non-stimulated B lymphocytes and also T lymphocytes all express PD-1, indicating that they are engaged in specific immune responses. PD-1, programmed death 1 is expressed in dendritic cells, macrophages and also monocytes, and is responsible for downregulating immune reponses. Some proinflammatory cytokines induce mRNA transcription of PD-1 in cytotoxic T lymphocytes and helper T lymphocytes. It interacts with the ligand, PD-L1 molecules transmitting lymphocyte inhibitory signal through PD-1. The PD-L1 molecule is mostly found on the surface of innate cells such as macrophages/ monocytes. PD-L1 is also expressed on tumor cells and this mechanism permits cancer cells to evade the immune system (Wojas-Krawczyk et al., 2019). In 2016, FDA gave approval to Atezolizumab, an anti-PD-1 monoclonal antibody, for the treatment of metastatic NSCLC patients whose disease deteriorated during platinum-containing chemotherapy or after this therapy. Atezolizumab was approved based on randomized clinical trials involving patients who have NSCLC. These trials demonstrated Atezolizumab's effectiveness as well as sound safety profile in these patients. From both clinical trials, Atezolizumab showed improvement in survival rate by 4.2 and 2.9 months compared to docetaxel. Patients with overexpression of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase tumor (ALKT) can begin treatment with Atezolizumab if other treatments fail (Alsaab et al., 2017). Nivolumab is a human monoclonal IgG4 and anti-PD-1 antibody. Phase III trial was conducted in 582 patients with advanced or metastatic squamous

cell NSCLC: 292 were given vaccine subcutaneously along with Nivolumab while the remaining 290 were given standard chemotherapy of docetaxel. The median overall survival for patients who were treated by Nivolumab was 12.2 months when compared to 9.4 months for patients who were treated by chemotherapy (Aldarouish & Wang, 2016). Pembrolizumab is a monoclonal antibody that targets PD-1. Pembrolizumab, like Nivolumab, has been very effective in treating melanoma and NSCLC (Sharon et al., 2014). BMS-936559 was the first IgG4 monoclonal antibody that targets PD-L1 to show effectiveness in NSCLC. BMS936559 Phase I clinical trial demonstrated 10% ORR within 49 patients who have NSCLC, also 12% of patients had long lasting disease at 6 months, PFS was 31% at 24 weeks. Several anti–PD-L1 antibodies have been found to be effective in NSCLC. A Phase I trial of MPDL-3280A, an anti-PD-L1 inhibitor, revealed a response rate (RR) of 21%. MEDI4736 is a humanized IgG1 monoclonal antibody, has been engineered to block PD-L1 to bind with PD-1, permitting T cells to acknowledge and eradicate cancer cells, MEDI4736 has been found to be active in non-small cell lung cancer (Anagnostou & Brahmer, 2015).

8.4 Adoptive Cell Therapy

Adoptive cell therapy is a kind of immunotherapy that includes isolation, identification, expansion and re-infusion of anti-tumor lymphocytes into patients. Adoptive cell therapy can be classified into tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy and chimeric antigen receptor (CAR) T cell therapy. In TIL therapy, T lymphocytes residing in tumors are isolated after surgical resection of the tumor, activated with the help of cytokines, expanded in the laboratory followed by reinfusion into the patients. On the contrary, in case of TCR and CAR T cell therapy, T cells are genetically modified to express T cell receptors (TCRs) or chimeric antigen receptors (CARs) specific to cancer antigens to carry out anti-tumor activity. To improve T cell survival, this type of treatment has been applied with the administration of proper growth factors (Yang et al., 2016).

Lymphokine-activated killer cells (LAKs) are a white blood cells, initially consisting of natural killer cells (NK), natural killer T cells (NKT) and also T cells (E. J. West et al., 2011). Patients are given genetically recombinant IL-2 (rIL-2) throughout the time of treatment to boost the power of LAK cells. Intrapleural shift of allogeneic LAK cells in combination with recombinant

IL-2 (rIL-2) were applied to treat 121 patients who have malignant effusion related to advanced lung cancer. In 71 patients, the effusion was eliminated and in 45 patients, the effusion was reduced without any harmful side effects noticed. In a clinical trial of 105 patients who went through non-curative resection of initial lung cancer were assigned into one group in total of two. The group of patients that received rIL-2 as well as LAK cells in combination with radiotherapy/ chemotherapy had shown a higher survival rate than the control group and this control group received radiation therapy or chemotherapy alone. A randomized Phase III study yielded a similar result. Due to the high levels of IL-2 connected to the clinical use of LAK cells, harmful side effects such as capillary leak syndrome, oliguria, pulmonary edema and also dyspnea have been indicated. These consequences are a significant impediment to the LAK cells advancement (Yang et al., 2016). Cytokine-induced killer (CIK) cells are immune effector cells with a mixture of T-NK phenotype including antitumor activity that is MHC-independent. Adoptive CIK cell transfer, one type of adoptive immunotherapy is a promising nontoxic anticancer treatment (Shi et al., 2013). In contrast, CIK cell efficacy has been very saddening in some clinical trials due to the reason for tumor cells escaping from the immune mediated destruction upgraded by CIK cells (Yu et al., 2011). One possible process of tumor resistance to cell therapy by using CIK is the abnormal microvasculature's hindrance of CIK cell infiltration into the area of tumor (T. L et al., 2014). CIK cell immunotherapy enhanced the effectiveness of chemotherapy in patients with advanced stage NSCLC, Phase II clinical study found (Yang et al., 2016). DC-activated CIK cells, which improved antitumor activity as well as chemotherapy in combination with CIK cells or with DC cells, enhanced outcomes in patients with advanced non-small cell lung cancer (Y. L et al., 2013). Combining CIK cells with the help of endostatin /DC-based cancer vaccines can exhibit a synergistic effect in clinical results. They have high proliferation rate and also cytotoxic activity, because of this, CIK cells are used popularly for several cancers treatment, particularly after activation by DCs (Z. R et al., 2014). NKT cells were discovered in 1986, and have characteristics of T cells as well as NK cells. In humans, natural killer T cells are distinguished with the expression of special changeless T-cell receptors which is encoded by the help of Vα24Jα18 (F. S et al., 2013). From a Phase I study of patients who have advanced and recurrent non-small cell lung cancer (n = 6), administration of activated NKT cells was very well accepted and performed carefully along with slight adverse reactions. From a clinical trial of invariant

natural killer T cell based immunotherapy, ligand pulsed infusion of APCs or iNKT cells that are activated, was found to be secured and well accepted (M. S et al., 2011).

Cytotoxic T lymphocytes (CTLs) are basically CD8+ T cells that serve as the strong power of anti-tumor immunity response. These can acknowledge MHC I molecules, present TAAs (tumor-associated antigens), and lyse tumor cells by releasing granzymes and perforins. As a result, techniques for increasing the amount of CTLs are now being advanced. Because of the downregulation, MHC molecules and also costimulatory molecules, re-infused CTLs are ineffective. There have been zero successful cases of CTL reinfusion therapy for lung cancer under these conditions. The identification of crucial factors, linked to the damage of MHC expression can point to a novel way for the advancement of immunotherapy options (Yang et al., 2016). TILs (tumor-infiltrating lymphocytes) are separated from tumors, draining lymph nodes or malignant effusion. TILs engineered by reinfusion of interleukin 2 gene into ten patients with developed lung cancer and also pleural effusions. In six patients, the pleural effusions did not show recurrence for four weeks and the original tumor size shrank in one patient (K et al., 2015). TIL re-infusion into the patients who have lung cancer has revealed several benefits, including good safety profile and specificity. Besides the advantages it has limitations which include the trouble in taking samples from surgeries and the negative effects connected to the combination of large-dose interleukin 2 as well as long culture periods (Yang et al., 2016).

8.4.1 Engineered T cell Therapy

Engineered T cell therapy targets tumor antigens and it is an appealing as well as potent cancer treatment option. Engineered T cell therapy for cancer may provide great progress to cancer immunotherapy with more laboratory changes as well as an increased amount of clinical study to examine these ways. Chimeric antigen receptor T (CAR-T) cells, that are basically genetically modified T cells, demonstrate CAR vector, which is designed to recognize and also bind with antigens on cancer cells (Qu et al., 2021). A CAR, which is an artificial fusion protein, contains extracellular antigen-binding domain, a transmembrane (TM) domain, and an intracellular signaling domain. CAR vector optimization can boost the safety as well as effectiveness of CAR-T cell therapy (M et al., 2020). Synthetic CARs have modified T lymphocytes to recognize particular tumor associated antigen from a variety of human cancerous tumors. The majority of

CAR-T-cell study in solid tumors is done focusing on NSCLC (Qu et al., 2021). The general approach in CAR-T-cell therapy is to use technology by transferring genes to reconfigure T cells of patients in order to demonstrate a CAR which is capable of binding to common antigens (CH et al., 2018). Peripheral blood T cells which are allogeneic are transfected along with the CAR complex (Rafiq et al., 2019). Prostate stem cell antigen (PSCA), EGFR and mesothelin and mucin 1, carcinoembryonic antigen, PD-L1, as well as human epidermal growth factor receptor 2 are the most targeted antigens of non-small cell lung cancer. Melanoma-associated antigen MAGE-A1, MAGE-A4, and Lewis-Y antigen are among several antigens being targeted with CAR-T cells in clinical study of the patients with lung cancer (Qu et al., 2021). However, T cells possess TCR on their surface which is a heterodimeric αβ receptor. Hence, antigenic peptides which is introduced by MHC proteins are recognized by this receptor. Genes encoding the TCRs α- and β-chains can be recognized as well as isolated from tumor-responsive patients' T Cells. Then, these chains are presented to T cells, typically using viral or non-viral vectors. Huge numbers of antigen-specific T cells are produced quickly in this manner. TCR-T cells that have been modified to respond to cancer cells that express the targeted antigen (Yang et al., 2016). TCR-T cell antigens are obtained from GP100, MART1, and NY-ESO-1, and MAGE family members and WT1 which are melanoma antigens. Clinical trials based on TCR-T cell therapy have shown significant effectiveness in melanoma. TCR-T cells, that recognize tumor antigens based on the HLA molecule. However TCRs must bind to CD3 molecules and are made up of transgenic α and β chains and chains. Also TCR-T cells can recognize antigens on tumor cells which are HLA-presented and attack them. The side effects of TCR-T cell therapy are reduced while it is compared to CAR T cell therapy. However, when the affinity or other properties of the TCR are altered, safety concerns should be prioritized. In preclinical and clinical trials of TCR-T cells generated very effective and strong immunity during the treatment of cancer and more patients should be enrolled to receive the benefits of TCR-T therapy (D. Li et al., 2019). The mechanism of engineered T cell therapy is depicted in Figure 4.

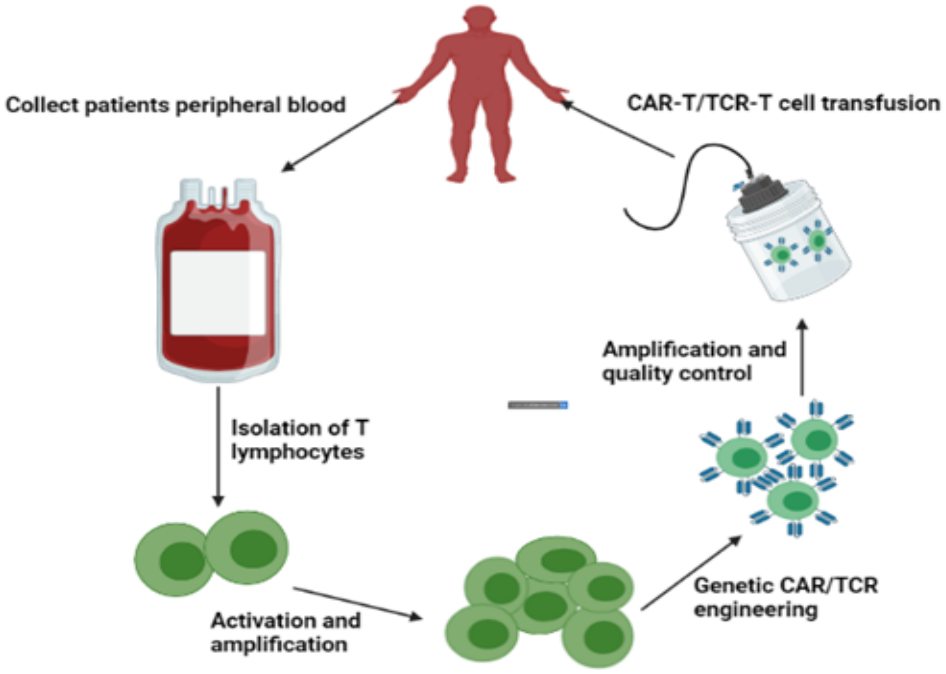

Figure 4: A flowchart of the process of engineered T cell therapy. A certain amount of patient's peripheral blood is collected for the development of engineered T cells. T cells are extracted from patient's peripheral blood mononuclear cells. Activation and amplification of T cells are done and T cells. The T cells are modified to express particular CARs or TCRs on the surface of the T cells. After completing the stages of amplification and quality control, CAR-T cells or TCR-T cells are infused back into the patient to enhance the antitumor activity (Adapted from (Zhao & Cao, 2019).

8.5 Anti-VEGF and Anti-EGFR Antibodies

Vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) are two other potential targets of immunotherapy. VEGF is involved in angiogenesis and EGFR assists in signal transduction for cell division and survival. Mutation in genes encoding for these two proteins have been linked to lung cancer in several studies. Bevacizumab (brand name Avastin), a humanized monoclonal antibody blocks the interaction of VEGF to its ligand, vascular endothelial growth factor receptor (VEGFR) expressed on the surface of endothelial cells. As a result, vascularization of tumor cells in disrupted. When administered with platinum-based chemotherapy, Bevacizumab has been found to significantly increase the overall survival

rate of advanced NSCLC patients (Alexander & Halmos, 2018). Mvasi (Amgen), the very first biosimilar to Avastin, got approval from FDA and EMA in the year of 2017 and 2018, respectively (bevacizumab-awwb). Following that, the EMA and FDA approved Zirabev (Pfizer), the second bevacizumab biosimilar, in 2019. Mvasi is approved for a variety of indications, which include metastatic non-small cell lung cancer (Konstantinidou et al., 2020). Ramucirumab has been also approved by FDA in 2020 in combination with erlotinib as first line treatment for metastatic EGFR-mutated NSCLC (FDA Approves Ramucirumab plus Erlotinib for First-Line Metastatic NSCLC | FDA, n.d.). Necitumumab and Mobocertinib, on the other hand, binds to EGFR and blocks tumor cell growth along with stimulating the immune system to kill the cells.

The list of FDA approved drugs used as immunotherapeutics for lung cancer are mentioned in Table 1.

Table 1: FDA approved drugs used in immunotherapy for lung cancer

Drug name (Target)	FDA approval year
Nivolumab (Anti-PD-1)	2015
Atezolizumab (Anti-PD-1)	2016
Pembrolizumab (Anti-PD-1)	2019
Durvalumab (Anti-PD-1)	2020
Avelumab (Anti-PD-L-1)	2017
Cemiplimab (Anti-PD-L-1)	2021
Bevacizumab (Anti VEGF-A)	2017
Ramucirumab (Anti VEGF-2)	2020
Necitumumab (Anti-EGFR)	2015
Mobocertinib (Anti- EGFR)	2021

Chapter 9

Challenges and Prospects of Cancer Immunotherapy

9.1 Challenges of Cancer Immunotherapy

The need for developing agents which are successful for major number patients and tumor types is a huge challenge for cancer immunotherapies. Patients who received cancer immunotherapies have seen dramatic improvements, denoting that it is possible to re - establish effective anti - tumor immune surveillance. Numerous immunotherapies have shown potency in just a selected group of tumors and in the few patients who were diagnosed with those particular cancers. Identification of further biomarkers and tumor pathways, tumor heterogeneity, variableness of tumor type and stage, history of treatment as well as cancer's immunosuppressive biology have all been proposed as rationale for changing patient reaction to cancer immunotherapies (Lee Ventola, 2017). Immunotherapies for cancer are not broadly recommended yet as first-line treatments; instead, they are administered to individuals whose immune systems are already dealt with advanced disease and previous therapies. The ability of cancer immunotherapies to reinstate antitumor immune responses is very challenging. Thus, large potency rates may be gained in a bigger proportion of patients if cancer immunotherapies which are personalized, were directed initially in order to reinstate a strong antitumor function whilst the immune response is yet recovering(H. West, 2014). One significant challenge of immunotherapy is the lack of targeted tumor-specific antigens (TSAs) also known as “neoantigens,” which are only demonstrated by cancer cells (Lee Ventola, 2017). Tumor-associated antigens which are demonstrated by cancer and normal tissues, are another immunotherapy drawback and targeting TAAS’s is probable to occur in off-target toxic reactions and showed little success. Because prognostic biomarkers predict patient results regardless of therapy used by the patients infrequently in taking decisions of treatments. Identifying biomarkers with predictive value used in choosing patients who will gain benefit from cancer immunotherapy, is a time-consuming as well as complicated method and very few prognostic biomarkers have been rigorously validated (J et al., 2016). Because cancer signaling networks are amazingly adaptable and flexible, resistance for some sole targeted treatment of cancer is probable. Cell population of subclonal cancer and also clonal evolution which are branched, are believed to play an important role in development of drug resistance

(Lee Ventola, 2017). In terms of overall survival and life quality, the addition of immunotherapeutics and novel targeted agents to the curative armamentarium is a real "game changer" for patients with cancer. Moreover, such agents are costly and the impacts of costs for the healthcare system must be considered carefully. Cancer treatment costs are high, thus close collaboration and coordination among the medical community, corporations, and economists are needed to ensure the global sustainability of health systems and patient care(F et al., 2016). The identification of new clinical and molecular prognostic biomarkers which may be used to choose patients to get advantage from high-priced targeted therapy and immunotherapy treatments may lower the costs. Assessments of cost-efficient, cost–utility, as well as quality adjustment of life and also various drug reimbursement modalities may also be helpful (Lee Ventola, 2017).

9.2 Prospects of Cancer Immunotherapy

Immunotherapies are an innovative way of treating lung cancer that has the possibility to provide long-term benefits even in advanced disease(Yang et al., 2016). In NSCLC, novel strategies involving immune checkpoint inhibitors, which have yielded hopeful initial outcomes as well as enduring reactions. In order to drive personalized curative decisions, it is crucial to identify the particular checkpoint pathways that operate immune resistance. Preclinical proof suggests transforming genes are responsible for antigen escape. Oncogenic EGFR signaling has been demonstrated to renovate the TME by upregulating immunosuppressive molecules which can regulate autoimmunity, such as PD-1, PD-L1, and CTLA-4. Oncogenic pathway inhibition may improve or discourage antitumor immunity providing justification for combined approaches incorporating molecularly targeted therapy as well as immunotherapy. Moreover, preparing endogenous response of tumor with cancer vaccines may cause immune checkpoints upregulation, which can be inhibited as a part of multi-modal therapy approaches. Immune checkpoint blockade, used in combination with newly invented molecules including anti-CD25 antibodies can consume CD4þFoxp3þ Treg cells, anti-CD27 antibodies can reverse CD8þ T-cell exhaustion when combined with PD-1 blockade as well as antibodies acknowledging the TNF superfamily receptor(Anagnostou & Brahmer, 2015). The outcomes of CTLA-4 and PD-1/PD-L1 suppression in patients who have melanoma sparked today's interest in cancer immunotherapy; however, some checkpoint molecules such as: TIM-3 and LAG-3, are being studied as a checkpoint inhibitor. TIM-3 inhibits activation of effector T cells, whilst LAG-3

inhibits activation of T cells as well as proliferation by binding to MHC molecules. Because LAG-3 co-expresses along with PD-1 in T cells, it has been a good nominee for the purpose of combination therapy along with anti-PD-1 agents. Antibodies, which are directed against TIM-3 and LAG-3 are being tested clinically and show promising results. Several other host immune targets are being studied in preclinical as well as clinical settings, such as inhibitory molecules (IDO1, B7-H3, B7-H4, VISTA, ICOS, KIR, and TIGIT) and stimulatory molecules (OX40, 4-1BB, and GITR) (M. R & TA, 2016). Novel immunotherapeutic agents that have demonstrated exceptional survival benefits when used in combination along with conventional therapies or alone show the possibility to change cancer into a life-long disease. This is currently the actual fact for patients with cancer who were given treatment with these agents. However, immunotherapeutic methods need to be improved further to increase potency while minimizing toxic reactions, which can be accomplished by designing clinical trials successfully. The field of immuno-oncology is developing rapidly, and the excitement should not fade, rather should expand in the upcoming years as novel potential strategies emerge (Tsiatas et al., 2016).

Chapter 10

Conclusion

Immunotherapy is a promising treatment for cancer that involves using the person's immune system to combat cancer and gaining attraction as a great complement to conventional treatments. Immune checkpoint blockade therapy, as well as vaccine therapy, have undoubtedly been the most significant advances in cancer therapeutics. Comprehensive efforts need to be made to identify and validate biomarkers that can predict, and immunotherapy using checkpoint blockade, combining with various immune therapeutic methods, is the major path for developing curative achievement over an extensive variety of tumors (Farkona et al., 2016). Knowledge of cancer immunology has developed dramatically in recent decades. Many challenges still continue to obstruct the accomplishment of immunotherapies for cancer in a broader range of diseases as well as patients. Leading to the current period, the fast growth of immunotherapy is anticipated to stay. It will be possible to overcome ongoing challenges by implementing obtainable and prospective solutions, such as: advancement of selected cancer immunotherapies, drug combinations of cancer immunotherapy for personalized treatment, techniques for cancer immunoprevention, as well as other significant changes (Lee Ventola, 2017).

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References

Alasadi, A. H., & M.ALsafy, B. (2015). Early Detection and Classification of Melanoma Skin Cancer. *International Journal of Information Technology and Computer Science*, *7*(12), 67–74. https://doi.org/10.5815/ijitcs.2015.12.08

Aldarouish, M., & Wang, C. (2016). Trends and advances in tumor immunology and lung cancer immunotherapy. *Journal of Experimental & Clinical Cancer Research*, 1–13. https://doi.org/10.1186/s13046-016-0439-3

Alexander, M., & Halmos, B. (2018). VEGF inhibitors in EGFR-mutated lung cancer: a never-ending story? *Annals of Translational Medicine*, *6*(23), 446–446. https://doi.org/10.21037/atm.2018.11.20

Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Frontiers in Pharmacology*, *8*(AUG), 1–15. https://doi.org/10.3389/fphar.2017.00561

Anagnostou, V. K., & Brahmer, J. R. (2015). Cancer immunotherapy: A future paradigm shift in the treatment of non-small cell lung cancer. *Clinical Cancer Research*, *21*(5), 976–984. https://doi.org/10.1158/1078-0432.CCR-14-1187

Araujo, L. H., Horn, L., Merritt, R. E., Shilo, K., Xu-Welliver, M., & Carbone, D. P. (2020). Cancer of the Lung. In *Abeloff's Clinical Oncology* (pp. 1108-1158.e16). Elsevier. https://doi.org/10.1016/B978-0-323-47674-4.00069-4

Baghban, R., Roshangar, L., Jahanban-Esfahlan, R., Seidi, K., Ebrahimi-Kalan, A., Jaymand, M.,

Kolahian, S., Javaheri, T., & Zare, P. (2020). Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling*, *18*(1), 1–19. https://doi.org/10.1186/s12964-020-0530-4

Bernhardt, E. B., & Jalal, S. I. (2016). Small cell lung cancer. In *Cancer Treatment and Research* (Vol. 170, pp. 301–322). Kluwer Academic Publishers. https://doi.org/10.1007/978-3-319-40389-2_14

Bertrand, A., Kostine, M., Barnetche, T., Truchetet, M. E., & Schaeverbeke, T. (2015). Immune related adverse events associated with anti-CTLA-4 antibodies: Systematic review and meta-analysis. *BMC Medicine*, *13*(1), 1–14. https://doi.org/10.1186/s12916-015-0455-8

Cancer Statistics - National Cancer Institute. (n.d.). Retrieved October 6, 2021, from https://www.cancer.gov/about-cancer/understanding/statistics

Canning, M., Guo, G., Yu, M., Myint, C., Groves, M. W., Byrd, J. K., & Cui, Y. (2019). Heterogeneity of the head and neck squamous cell carcinoma immune landscape and its impact on immunotherapy. *Frontiers in Cell and Developmental Biology*, *7*(APR), 1–19. https://doi.org/10.3389/fcell.2019.00052

CH, J., RS, O., OU, K., S, G., & MC, M. (2018). CAR T cell immunotherapy for human cancer. *Science (New York, N.Y.)*, *359*(6382), 1361–1365. https://doi.org/10.1126/SCIENCE.AAR6711

Chew, V., Toh, H. C., & Abastado, J. P. (2012). Immune microenvironment in tumor progression: Characteristics and challenges for therapy. *Journal of Oncology*, *2012*. https://doi.org/10.1155/2012/608406

Collins, L. G., Haines, C., Perkel, R., & Enck, R. E. (2007). Lung cancer: Diagnosis and management. *American Family Physician*, *75*(1), 56–63.

Declerck, S., & Vansteenkiste, J. (2014). *Immunotherapy for lung cancer : ongoing clinical trials*. *10*, 91–105.

Dela Cruz, C. S., Tanoue, L. T., & Matthay, R. A. (2011). Lung Cancer: Epidemiology, Etiology, and Prevention. *Clinics in Chest Medicine*, *32*(4), 605–644. https://doi.org/10.1016/j.ccm.2011.09.001

Domingues, D., Turner, A., Silva, M. D., Marques, D. S., Mellidez, J. C., Wannesson, L., Mountzios, G., & De Mello, R. A. (2014). Immunotherapy and lung cancer: Current developments and novel targeted therapies. *Immunotherapy*, *6*(11), 1221–1235. https://doi.org/10.2217/imt.14.82

F, T., M, S., L, B., P, M., A, O., & R, B. (2016). Economic sustainability of anti-PD-1 agents nivolumab and pembrolizumab in cancer patients: Recent insights and future challenges. *Cancer Treatment Reviews*, *48*, 20–24. https://doi.org/10.1016/J.CTRV.2016.06.002

Farkona, S., Diamandis, E. P., & Blasutig, I. M. (2016). the end Cancer immunotherapy : the beginning of of cancer ? *BMC Medicine*, 1–18. https://doi.org/10.1186/s12916-016-0623-5

FDA approves ramucirumab plus erlotinib for first-line metastatic NSCLC | FDA. (n.d.). Retrieved October 27, 2021, from https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-ramucirumab-plus-erlotinib-first-line-metastatic-nsclc

Germ cell tumours | Cancer Research UK. (n.d.). Retrieved May 29, 2021, from

https://www.cancerresearchuk.org/about-cancer/germ-cell-tumours

Hanna, M. G., Howard, J., & Vermorken, J. (2014). Active specific immunotherapy: Using tumor heterogeneity to successfully fight cancer. *Human Vaccines and Immunotherapeutics*, *10*(11), 3286–3296. https://doi.org/10.4161/hv.28886

Hassan Lemjabbar-Alaouia, O. H., Yanga, Y.-W., & Buchanana, P. (2017). Lung cancer: biology and treatment options. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1016/j.bbcan.2015.08.002.Lung

Howington, J. A., Blum, M. G., Chang, A. C., Balekian, A. A., & Murthy, S. C. (2013). Treatment of stage I and II non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American college of chest physicians evidence-based clinical practice guidelines. *Chest*, *143*(5 SUPPL), e278S-e313S. https://doi.org/10.1378/chest.12-2359

Immune Checkpoint Inhibitors - National Cancer Institute. (n.d.). Retrieved October 29, 2021, from https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

J, Z., C, G., S, P., I, F., S, M.-P., & L, P.-A. (2016). Current Challenges in Cancer Treatment. *Clinical Therapeutics*, *38*(7), 1551–1566. https://doi.org/10.1016/J.CLINTHERA.2016.03.026

Joshi, M., Ayoola, A., & Belani, C. P. (2013). Small-cell lung cancer: An update on targeted therapies. In *Advances in Experimental Medicine and Biology* (Vol. 779, pp. 385–404). Springer Science and Business Media, LLC. https://doi.org/10.1007/978-1-4614-6176-0_18

K, L., Q, Z., Y, Z., J, Y., & J, Z. (2015). T-cell-associated cellular immunotherapy for lung cancer. *Journal of Cancer Research and Clinical Oncology*, *141*(7), 1249–1258. https://doi.org/10.1007/S00432-014-1867-0

Karim, N. A., Schuster, J., Eldessouki, I., Gaber, O., Namad, T., Wang, J., Xie, C., & Morris, J. C. (2018). Pulmonary sarcomatoid carcinoma: University of Cincinnati experience. *Oncotarget*, *9*(3), 4102–4108. https://doi.org/10.18632/oncotarget.23468

Kokate, R. (2017). A Systematic Overview of Cancer Immunotherapy: An Emerging Therapy. *Pharmacy & Pharmacology International Journal*, *5*(2), 31–35. https://doi.org/10.15406/ppij.2017.05.00112

Konstantinidou, S., Papaspiliou, A., & Kokkotou, E. (2020). Current and future roles of biosimilars in oncology practice (Review). *Oncology Letters*, *19*(1), 45–51. https://doi.org/10.3892/ol.2019.11105

L, T., G, H., S, S., & L, C. (2014). Bevacizumab improves the antitumor efficacy of adoptive cytokine-induced killer cells therapy in non-small cell lung cancer models. *Medical Oncology (Northwood, London, England)*, *31*(1). https://doi.org/10.1007/S12032-013-0777-3

L, Y., B, R., H, L., J, Y., S, C., X, H., & X, R. (2013). Enhanced antitumor effects of DC-activated CIKs to chemotherapy treatment in a single cohort of advanced non-small-cell lung cancer patients. *Cancer Immunology, Immunotherapy : CII*, *62*(1), 65–73. https://doi.org/10.1007/S00262-012-1311-8

Lakna. (2017). *Difference Between Humoral and Cell Mediated Immunity | Definition,*

Characteristics, How They Act. *September*. https://pediaa.com/difference-between-humoral-and-cell-mediated-immunity/

Lee Ventola, C. (2017). Cancer immunotherapy, part 3: Challenges and future trends. *P and T*, *42*(8), 514–521. https://doi.org/10.1007/978-3-319-17900-1_176

Li, C., & Lu, H. (2018). Adenosquamous carcinoma of the lung. *OncoTargets and Therapy*, *11*, 4829–4835. https://doi.org/10.2147/OTT.S164574

Li, D., Li, X., Zhou, W. L., Huang, Y., Liang, X., Jiang, L., Yang, X., Sun, J., Li, Z., Han, W. D., & Wang, W. (2019). Genetically engineered t cells for cancer immunotherapy. *Signal Transduction and Targeted Therapy*, *4*(1). https://doi.org/10.1038/s41392-019-0070-9

Lovly, C. M., Li, C.-I., Hutchison, A. S., Carbone, D. P., Johnson, D. H., Keedy, V. L., Pao, W., Sandler, A., & Horn, L. (2012). A phase II study of second-line bendamustine in relapsed or refractory small cell lung cancer (SCLC). *Journal of Clinical Oncology*, *30*(15_suppl), 7094–7094. https://doi.org/10.1200/jco.2012.30.15_suppl.7094

Lung cancer - Treatment - NHS. (n.d.). Retrieved June 4, 2021, from https://www.nhs.uk/conditions/lung-cancer/treatment/

M, M., E, A., J, R., C, H., M, K., N, B., B, O., L, L., GJ, R., ML, G., AM, M., S, W., & CE, M. (2020). The therapeutic landscape for cells engineered with chimeric antigen receptors. *Nature Biotechnology*, *38*(2), 233–244. https://doi.org/10.1038/S41587-019-0329-2

Marcus, A., Gowen, B. G., Thompson, T. W., Iannello, A., Ardolino, M., Deng, W., Wang, L., Shifrin, N., & Raulet, D. H. (2014). Recognition of tumors by the innate immune system

and natural killer cells. In *Advances in Immunology* (Vol. 122). https://doi.org/10.1016/B978-0-12-800267-4.00003-1

Marrugal, Á., Ojeda, L., Paz-Ares, L., Molina-Pinelo, S., & Ferrer, I. (2018). Corrigendum to "Proteomic-Based Approaches for the Study of Cytokines in Lung Cancer." *Disease Markers*, *2018*, 1404780. https://doi.org/10.1155/2018/1404780

Massarelli, E., Papadimitrakopoulou, V., Welsh, J., Tang, C., & Tsao, A. S. (2014). Immunotherapy in lung cancer. *Translational Lung Cancer Research*, *3*(1), 53–63. https://doi.org/10.3978/j.issn.2218-6751.2014.01.01

Mesothelioma - NHS. (n.d.). Retrieved July 16, 2021, from https://www.nhs.uk/conditions/mesothelioma/

Mesothelioma | Causes, Symptoms & Treatments | Cancer Council. (n.d.). Retrieved June 5, 2021, from https://www.cancer.org.au/cancer-information/types-of-cancer/mesothelioma

Monjazeb, A. M., Hsiao, H. H., Sckisel, G. D., & Murphy, W. J. (2012). The role of antigen-specific and non-specific immunotherapy in the treatment of cancer. *Journal of Immunotoxicology*, *9*(3), 248–258. https://doi.org/10.3109/1547691X.2012.685527

Multiple myeloma - NHS. (n.d.). Retrieved June 2, 2021, from https://www.nhs.uk/conditions/multiple-myeloma/

Neuroendocrine tumours - NHS. (n.d.). Retrieved May 29, 2021, from https://www.nhs.uk/conditions/neuroendocrine-tumours/

Non Small Cell Lung Cancer - PubMed. (n.d.). Retrieved June 3, 2021, from

https://pubmed.ncbi.nlm.nih.gov/32965978/

Osipov, A., Murphy, A., & Zheng, L. (2019). From immune checkpoints to vaccines: The past, present and future of cancer immunotherapy. In *Advances in Cancer Research* (Vol. 143, pp. 63–144). Academic Press Inc. https://doi.org/10.1016/bs.acr.2019.03.002

Qu, J., Mei, Q., Chen, L., & Zhou, J. (2021). Chimeric antigen receptor (CAR)-T-cell therapy in non-small-cell lung cancer (NSCLC): current status and future perspectives. *Cancer Immunology, Immunotherapy*, *70*(3), 619–631. https://doi.org/10.1007/s00262-020-02735-0

R, M., & TA, C. (2016). Personalized Oncology Meets Immunology: The Path toward Precision Immunotherapy. *Cancer Discovery*, *6*(7), 703–713. https://doi.org/10.1158/2159-8290.CD-16-0146

R, Z., B, H., & H, Z. (2014). A prospective study of the efficacy of a combination of autologous dendritic cells, cytokine-induced killer cells, and chemotherapy in advanced non-small cell lung cancer patients. *Tumour Biology : The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *35*(2), 987–994. https://doi.org/10.1007/S13277-013-1132-1

Rafiq, S., Hackett, C. S., & Brentjens, R. J. (2019). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nature Reviews Clinical Oncology 2019 17:3*, *17*(3), 147–167. https://doi.org/10.1038/s41571-019-0297-y

S, F., K, S., Y, O., N, K., T, N., S, M., & M, T. (2013). NKT cells as an ideal anti-tumor immunotherapeutic. *Frontiers in Immunology*, *4*(DEC). https://doi.org/10.3389/FIMMU.2013.00409

S, M., Y, O., I, Y., & T, N. (2011). Anti-tumor immune responses induced by iNKT cell-based immunotherapy for lung cancer and head and neck cancer. *Clinical Immunology (Orlando, Fla.)*, *140*(2), 167–176. https://doi.org/10.1016/J.CLIM.2011.01.009

Saxena, M., van der Burg, S. H., Melief, C. J. M., & Bhardwaj, N. (2021). Therapeutic cancer vaccines. *Nature Reviews Cancer*, *21*(6), 360–378. https://doi.org/10.1038/s41568-021-00346-0

Shi, S., Wang, R., Chen, Y., Song, H., Chen, L., & Huang, G. (2013). Combining Antiangiogenic Therapy with Adoptive Cell Immunotherapy Exerts Better Antitumor Effects in Non-Small Cell Lung Cancer Models. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065757

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer Statistics, 2021. *CA: A Cancer Journal for Clinicians*, *71*(1), 7–33. https://doi.org/10.3322/caac.21654

Spurrell, E. L., & Lockley, M. (2014). Adaptive immunity in cancer immunology and therapeutics. *Ecancermedicalscience*, *8*(1), 1–10. https://doi.org/10.3332/ecancer.2014.441

Steven, A., Fisher, S. A., & Robinson, B. W. (2016). Immunotherapy for lung cancer. *Respirology*, *21*(5), 821–833. https://doi.org/10.1111/resp.12789

Sun, Y. (2017). Immunosurveillance and immunotherapy of tumors by innate immune cells. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1016/j.coi.2015.11.001.Immunosurveillance

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F.

(2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Symptoms of Cancer - National Cancer Institute. (n.d.). Retrieved May 29, 2021, from https://www.cancer.gov/about-cancer/diagnosis-staging/symptoms

Tsiatas, M., Mountzios, G., & Curigliano, G. (2016). Future perspectives in cancer immunotherapy. *Annals of Translational Medicine*, *4*(14). https://doi.org/10.21037/atm.2016.07.14

Types of cancer | Cancer Research UK. (n.d.). Retrieved June 2, 2021, from https://www.cancerresearchuk.org/what-is-cancer/how-cancer-starts/types-of-cancer

Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., Yokoyama, W. M., & Ugolini, S. (2011). Innate or adaptive immunity? The example of natural killer cells. *Science*, *331*(6013), 44–49. https://doi.org/10.1126/science.1198687

Wang, Y., Hays, E., Rama, M., & Bonavida, B. (2020). Cell-mediated immune resistance in cancer. *Cancer Drug Resistance*, *3*(2), 232–251. https://doi.org/10.20517/cdr.2019.98

West, E. J., Scott, K. J., Jennings, V. A., & Melcher, A. A. (2011). Immune activation by combination human lymphokine-activated killer and dendritic cell therapy. *British Journal of Cancer*, *105*(6), 787–795. https://doi.org/10.1038/bjc.2011.290

West, H. (2014). Nivolumab as first line monotherapy for advanced non-small cell lung cancer: Could we replace first line chemotherapy with immunotherapy? *Translational Lung Cancer*

Research, *3*(6), 400–402. https://doi.org/10.3978/j.issn.2218-6751.2014.09.04

What Is Cancer? - National Cancer Institute. (n.d.). Retrieved May 26, 2021, from https://www.cancer.gov/about-cancer/understanding/what-is-cancer

Whiteside, T. L. (2008). The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008 Oct;27(45):5904.ss. *Oncogene*, *27*(45), 5904–5912. https://doi.org/10.1038/onc.2008.271.The

Winter, H., van den Engel, N. K., Rusan, M., Schupp, N., Poehlein, C. H., Hu, H. M., Hatz, R. A., Urba, W. J., Jauch, K. W., Fox, B. A., & Rüttinger, D. (2011). Active-specific immunotherapy for non-small cell lung cancer. *Journal of Thoracic Disease*, *3*(2), 105–114. https://doi.org/10.3978/j.issn.2072-1439.2010.12.06

Wojas-Krawczyk, K., Kalinka, E., Grenda, A., Krawczyk, P., & Milanowski, J. (2019). Beyond PD-L1 markers for lung cancer immunotherapy. *International Journal of Molecular Sciences*, *20*(8). https://doi.org/10.3390/ijms20081915

Yang, L., Wang, L., & Zhang, Y. (2016). Immunotherapy for lung cancer: advances and prospects. *American Journal of Clinical and Experimental Immunology*, *5*(1), 1. /pmc/articles/PMC4858602/

Yu, J., Ren, X., Li, H., Cao, S., Han, Y., Enoki, T., Kato, I., Cao, C., & Hao, X. (2011). Synergistic Effect of CH-296 and Interferon Gamma on Cytokine-Induced Killer Cells Expansion for Patients with Advanced-Stage Malignant Solid Tumors. *Https://Home.Liebertpub.Com/Cbr*, *26*(4), 485–494. https://doi.org/10.1089/CBR.2010.0927

Yuan, C., Keating, B., Farricielli, L. A., & Zhang, K. (2014). Large-cell neuroendocrine carcinoma (LCNEC) without pulmonary symptoms diagnosed in a cutaneous metastasis. *American Journal of Case Reports*, *15*, 97–102. https://doi.org/10.12659/AJCR.890094

Zappa, C., & Mousa, S. A. (2016). Non-small cell lung cancer: Current treatment and future advances. *Translational Lung Cancer Research*, *5*(3), 288–300. https://doi.org/10.21037/tlcr.2016.06.07

Zhao, L., & Cao, Y. J. (2019). Engineered T Cell Therapy for Cancer in the Clinic. *Frontiers in Immunology*, *10*(October). https://doi.org/10.3389/fimmu.2019.02250