A review on the tumour immune microenvironment andthe emergence of mRNA vaccines as a novel therapeuticstrategy

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***Abstract*- Cancer is the second leading cause of death in the world. The immune system has been shown to be a critical regulator of tumour development through clearance and via the process of immunoediting, by which cancers will dampen the immune response to promote their progression. The advent of immunotherapies has become a revolutionary treatment as it uses the nature of the immune system to fight back at cancers. This review will focus on the pivotal role of the tumour microenvironment in tumour progression and tumour-immune dynamics, through cellular signalling, gene expression and cell-cell interactions. Subsequently, an overview of current immunotherapies will be presented and evaluated. Finally, this review will present mRNA vaccines as a novel therapeutic strategy and determine their implications for cancer treatment.**

I. INTRODUCTION

Global Cancer Burden

Cancer is a broad term that refers to a range of disorders characterised by abnormal cell growth and division. The illness is intricate and multidimensional, and it can affect any region of the body. Among other organs and tissues, cancer can develop in the lungs, breast, colon, prostate, and skin. Cancer is a leading cause of death worldwide (figure 1), accounting for nearly 10 million deaths in 2020 [1]. Breast and lung cancers were the most common cancers worldwide, contributing 12.5% and 12.2% of the total number of new cases diagnosed in 2020 [2].

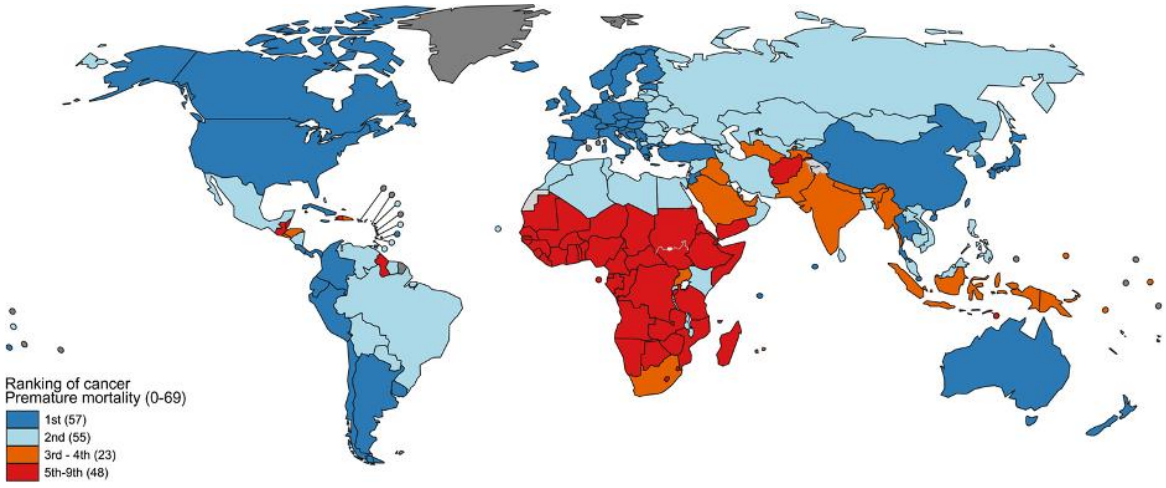

Figure 1. A global map indicating the levels of premature cancer mortality experienced in the different regions of the world as of 2020. Figure adapted from [3].

Researching cancer is important for a number of reasons. It contributes to our understanding of the condition, advances early detection, improved therapy development, lowers risk factors, improves patient care, and addresses cancer's worldwide effects. Understanding the causes, mechanisms, and behaviour of cancer through research paves the way for improvements in its detection, diagnosis, and treatment. We can improve outcomes, raise survival rates, and improve patient and family quality of life by studying cancer.

The Hallmarks of Cancer

Cancer is a disease in which cells proliferate uncontrollably and develop to other parts of the body. Specific treatments have been developed to target elements of the tumour required for growth and survival[38]. These

dependencies are termed the 'hallmarks of cancer' (figure 2) [4].

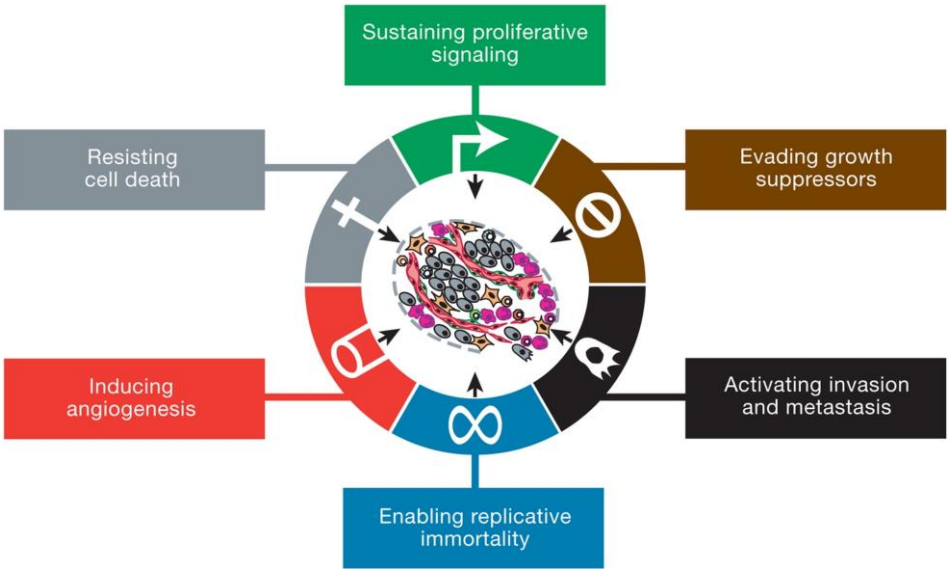

Figure 2. An overview of the hallmarks of cancer. Adapted from [4]

Sustained proliferative signalling:
Cancers continuously receive and send signals that promote unchecked growth and division. Genetic mutations can disrupt the harmony of growth factors, proto-oncogenes and tumour suppressor genes. The overproduction of growth factors or increased receptor sensitivity brought on by mutations can make cancer cells more susceptible to growth signals than healthy cells are. For example, mutations in the epidermal growth factor receptor (EGFR), HER2 [5].

Cancer cells may also use feedback loops and epigenetic modifications to boost signals that promote growth and get around controls on cell division. The epigenetic landscape of cancers include histone modifications, DNA methylation, changes in chromatin accessibility and regulation of non-coding RNAs. Such misregulation of signalling pathways allows cancer cells to multiply uncontrollably, leading to tumour formation and advancing malignancy[39].

Evading growth suppressors:
Cancers have the ability to go around the built-in controls that prevent unhealthy cells from dividing and growing too quickly. Checkpoints in healthy cells control the cell cycle to ensure that growth and division only occur when the right signals are received and the cellular environment is favourable for healthy growth [4].

However, cancer cells evolve a number of evasion mechanisms to circumvent these growth-suppressing systems. First, tumour suppressor genes such as retinoblastoma protein (pRB), which usually control cell proliferation, can have mutations or deletions in cancer cells [4]. The restriction on cell proliferation is removed when these genes are lost.

Second, oncogene hyperactivation. Oncogenes stimulate cell proliferation when they are mutated or overexpressed. These signals may be amplified by cancer cells, which would promote unchecked cell division. Normal cells halt the cell cycle to repair damaged DNA when DNA is damaged. Cancer cells that have ineffective DNA repair systems continue to grow despite DNA damage. Cancer cells have the ability to avoid the genetic mutations and programmed cell death (apoptosis) that are brought on by cellular abnormalities[40].

Resisting Cell Death:
Apoptosis, or programmed cell death usually eliminates damaged or useless cells. However, cancer cells have the characteristic of resisting apoptosis. Cancer cells use a variety of methods to avoid apoptosis. First, they can directly upset apoptosis by upsetting the balance of pro- and anti-apoptotic proteins such as the Bcl-2 family [6]. Second, cancers can mutate tumour suppressor genes such as p53 to reduce its capacity to trigger apoptosis in response to cellular stress or DNA damage [7]. Third, tumours often activate survival pathways, for instance those that support cell survival and prevent apoptosis, such as PI3K/AKT and MAPK [8]. Finally, cancer cells change the death receptors on their surface, which makes them less sensitive to apoptotic signals. For example, they will mutate the tumour necrosis factor receptor (TNFR) [9].

Enabling replicative immortality:
Cancers often induce replicative immortality by activation of human telomerase reverse transcriptase (hTERT), which lengthens the telomeres, the protective caps at the ends of chromosomes. When telomeres in normal cells reach a certain length, cell senescence and growth arrest occur. Telomeres in normal cells decrease during each cell division. hTERT is turned on in cancer cells, preventing telomere shortening and allowing unabated cell division [10].

Alternative Lengthening of Telomeres (ALT) is a telomerase-independent method that some cancer cells use to retain their capacity for replication. Replicative immortality must be enabled in order for cancer to advance since it permits unchecked proliferation, tumour growth, and potential metastasis [10].

Inducing Angiogenesis:
The ability of cancer cells to promote the creation of new blood vessels (neo-angiogenesis) in order to sustain their growth and collect vital nutrients for tumour progression is another hallmark. Pro-angiogenic substances released by cancer cells, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), stimulate endothelial cells and adjacent blood arteries [11]. These dividing, migrating activated endothelial cells create new capillaries that join the tumour to the host's circulatory system. This mechanism enables the tumour 's continuous development and expansion by supplying it with oxygen, nutrients, and growth-promoting agents.

Additionally, angiogenesis is essential for the metastasis of cancer, allowing the bloodstream transfer of cancer cells to distant places. For a tumour to survive, develop, and progress, cancer cells must be able to trigger angiogenesis.

Activation invasion and metastasis:
Cancer cells develop the capacity to infect neighbouring tissues, get inside the circulation or lymphatic system, and then travel to distant regions of the body, where they might metastasize or create additional tumours. In order to facilitate invasion and metastasis, the cancer cells first have to break down their surrounding extracellular matrix (ECM) to create passages towards the lymphatic or circulatory systems. Therefore cancer cells will secrete ecrete enzymes called matrix metalloproteinases (MMPs), which degrade ECM components that provide structural support to tissues such as collagens and fibrinogens. ECM degradation facilitates cancer cell movement through the tissue barriers, promoting invasion into nearby blood vessels or lymphatics [12].

The majority of cancer-related deaths are caused by metastatic cancer, which is extremely difficult to treat. The survival and proliferation of metastatic cancer cells are aided by their adaptation to survive in circulation as circulating tumour cells (CTCs) and to establish novel microenvironments in distant organs. Metastatic tumours frequently develop resistance to common therapies as a result of these changes, creating treatment difficulties[13].

Another common mechanism for cancer metastasis is to transform into a more plastic and motile state. This process is known as epithelial-to-mesenchymal transition (EMT). During EMT, cancer cells lose cell-to-cell adhesions such as E-cadherin and adopt a more fibroblast-like state [13] Furthermore EMT promotes survival of anoikis (programmed cell death for cells that lose adhesion to their surrounding environment), thus surviving in circulation [13].

II. THE TUMOUR MICROENVIRONMENT AND THE IMMUNE SYSTEM

The Tumour Microenvironment

The tumour microenvironment (TME) is an innervated environment consisting of proliferating tumour cells, the tumour stroma, blood vessels, immune cells such as infiltrating T cells and a variety of associated tissue cells such as mesenchymal cells (figure 3). The cells within the TME contribute on a physiological level to the progression of cancer. For example, endothelial progenitor cells participate in angiogenesis, the process of forming new blood vessels that supply oxygen and nutrients to enable the development of tumours [11].

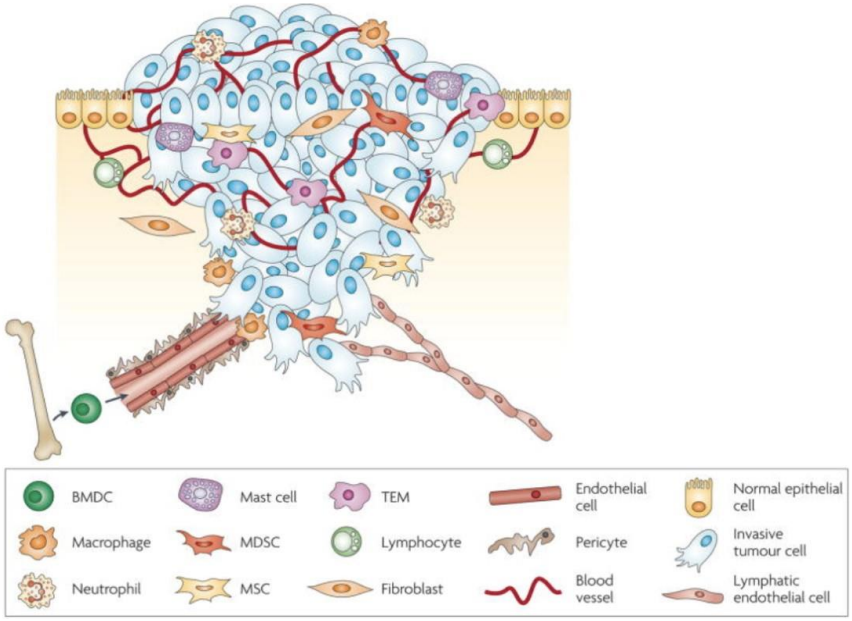

Figure 3. Schematic of the tumour microenvironment. Adapted from [14].

Of interest to this paper, immune cells can play dual and opposing roles in the TME. For instance, T cells are a type of white blood cells that determine the particular immune response to antigens that have entered the body. T cells are formed in the bone marrow and consist of particular types of cells include: Helper T cells, Killer/Cytotoxic T cells, and Memory T cells. However, cytotoxic T cells can also recognize cancer cells and destroy them. Therefore a high proportion of infiltrating T cells will be detrimental to the tumour (see later).

In contrast, macrophages, another type of white blood cell, are frequently attracted to and polarised into pro-tumorigenic phenotypes in the primary tumour microenvironment [15]. These tumour-associated macrophages encourage the migration, invasion, and intravasation of tumour cells into the bloodstream.

Another element of the TME, the population of cells known as mesenchymal stem cells (MSCs), are adaptable and capable of differentiating into numerous cell types. MSCs are attracted to primary tumors and help to increase metastasis by secreting growth factors and cytokines that assist tumour cell survival, migration, and invasion [16].

Finally, platelets are essential to the TME as they shield tumour cells from immunological attack and physical damage as they circulate throughout the body.

Immunoediting

Cancer immunoediting is the process in which the immune system restrains the and eventually promotes the tumour development in three main stages: Elimination, Equilibrium and Escape (figure 4).

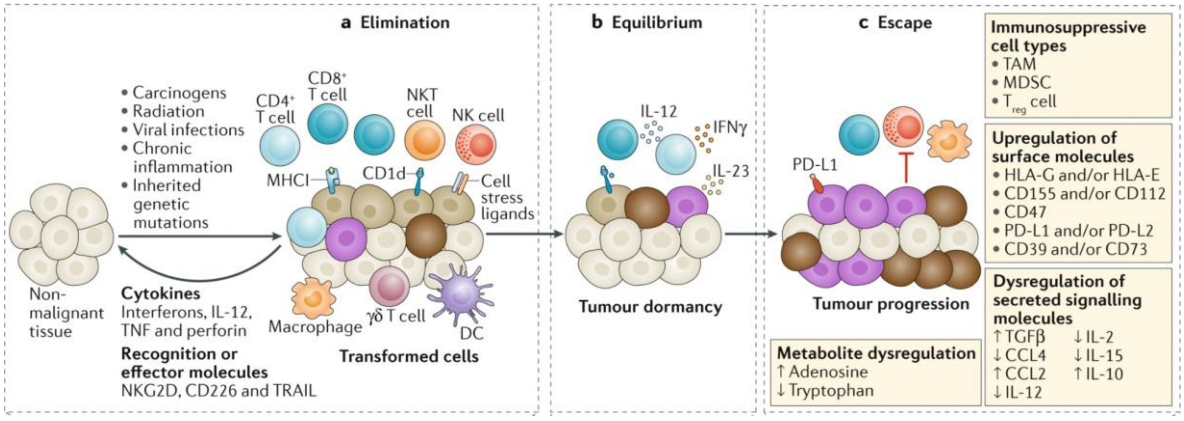

Figure 4. An overview of cancer immunoediting. Adapted from [17].

The first step of immune response is the "elimination" phase, during which the immune system locates and destroys

cancer cells. Cytotoxic immune cells, such as T cells and natural killer (NK) cells, hunt for and eliminate malignant cells during this phase[17]. This essential defence system aids in preventing the growth of cancer.
However, some cancer cells may create strategies to avoid immune detection and eradication. Cancer cells that have specific genetic or chemical changes may be more vulnerable to immune response during the elimination phase, which results in their elimination before they can form a tumour[17]. This explains why many tumours never develop past the early stages. But some cancer cells might experience alterations that let them avoid the immune response and reach the "equilibrium" phase.

The second stage of immunoediting is equilibrium during this stage, cancer cells that have not yet been entirely eradicated by the immune system are in a condition of equilibrium with the immunological response. The immune system has some degree of control over the development and spread of cancer cells during this stage, preventing them from becoming a clinically discernible tumour[17]. These cancer cells can still exist in a latent or low-level state and are not completely eliminated. In the equilibrium phase, immunotherapy tries to optimise and improve the immune response against these persistent cancer cells. The objective is to increase the immune system's capacity to identify and effectively combat cancer cells in order to maintain or reinforce the equilibrium condition. Researchers have come up with immunotherapies that could be used in the equilibrium state to eradicate cancer like Adoptive T-cell therapy, Checkpoint inhibitors, Cancer Vaccines etc.

The final stage of immunoediting is escape which describes the stage of cancer development and progression where cancer cells that have managed to elude the immune system's surveillance and control mechanisms grow and spread uncontrollably, eventually resulting in the formation of a clinically detectable tumour. The cancer cells have developed a number of strategies during this stage that enable them to outwit the immune system and avoid being destroyed[17].

Immune Evasion

Immune evasion is a strategy used by pathogenic organisms and tumours to evade a host's immune response to maximize their probability of being transmitted to a fresh host or to continue growing, respectively [18].

Macrophages are immune cells which play a vital role in homeostatic maintenance of the body through the disposal of internal waste and tissue repair. There are two types of macrophages, M1 and M2 [19]. The immune system, tumour cells, and the local microenvironment all play important roles in the link between M2 macrophages and cancer evasion. Shifting the balance of immune cells within the TME helps contribute to immune evasion. Cancer cells will preferentially accumulate M2 macrophages over M1 macrophages. M1 macrophages secrete cytokines such as IL-12 and IFNɣ, leading to activation of other immune cells and an antitumor response. However, M2 macrophages secrete VEGF, MMP1 and FGF2, promoting angiogenesis, degradation of the ECM and proliferation, respectively [19]. Thus, the M2-like tumour associated macrophages can paradoxically drive tumour progression and metastasis, while simultaneously evading the immune system.

The following are some ways that M2 macrophages can aid in cancer evasion: M2 macrophages can secrete cytokine) suppression of Cytotoxic T cells, which are in charge of identifying and killing cancer cells, are activated by cytokines and other substances that M2 macrophages can release [20]. The body's capacity to generate an efficient immune response against the tumour may be hampered by this immunosuppressive environment created. Angiogenesis is encouraged by substances that M2 macrophages can produce, which help the tumour's existing blood arteries create new ones. This procedure aids in supplying the tumour with nutrition and oxygen, enabling it to develop and avoid immune surveillance. M2 macrophages play a part in tissue remodelling and healing as well as immune evasion [20]. They can aid in establishing conditions that prevent tumour cells from being recognised by the immune system in the context of cancer. This includes the creation of elements of the extracellular matrix that act as a physical barrier to keep immune cells from getting to the tumour cells. M2 macrophages have the ability to create and secrete cytokines and chemokines, which draw in immune cells with immunosuppressive qualities [20]. In turn, these immune cells may contribute to the development of a setting that dampens the anti-tumor immune response.

Immune Suppression

Tumour cells can use a variety of tactics to evade immune detection and establish an immunosuppressive TME. Together, these tactics reduce the immune response and promote the growth of tumours. For example, multiple tumour-suppressing chemicals, including IL-10, TGF-, prostaglandin E2, and VEGF, are released by tumour cells. These chemicals help to produce an immune-suppressive TME that prevents immune cell activation and encourages immunological tolerance [20].

In addition, human leukocyte antigens (HLAs) on the surface of tumour cells can be downregulated, which decreases

their capacity to deliver antigens to cytotoxic T lymphocytes [21]. As a result, the immune system is less likely to recognise them. Immune evasion and resistance to immune-based treatments have been associated with loss of HLA expression. Tumour cells have the ability to suppress cell surface chemicals that stimulate natural killer (NK) cells during metastasis. By doing this, the tumour cells are able to avoid being noticed by NK cells, which are a component of the innate immune response [21].

Checkpoint inhibitory ligands or receptors including PD-L1, CTLA-4, and VISTA can be expressed by tumour cells [21]. These proteins interact with immune cells, especially T cells, and reduce their activity. As a result, the immune system is unable to effectively combat the tumour. Tumour cells secrete chemokines that draw immune-suppressive cells such regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumour-associated macrophages (TAMs). These immune cells also impair anti-tumor immune responses while adding to the immunosuppressive environment [21].

The degree to which the immune system and the cancer TME interact can be classified into “hot” or “cold” tumours (figure 5). Melanoma and lung cancer demonstrate high response rates to immune checkpoint inhibitors and are commonly referred to as hot tumours, while prostate and pancreatic cancers are often cold tumours. A tumour that has a significant immune cell infiltration is referred to as a "hot" tumour. Immune cells, including T cells and other immune effectors, are located inside and around a heated tumour. In contrast, a "cold" tumour does not have a considerable immune cell invasion [22].

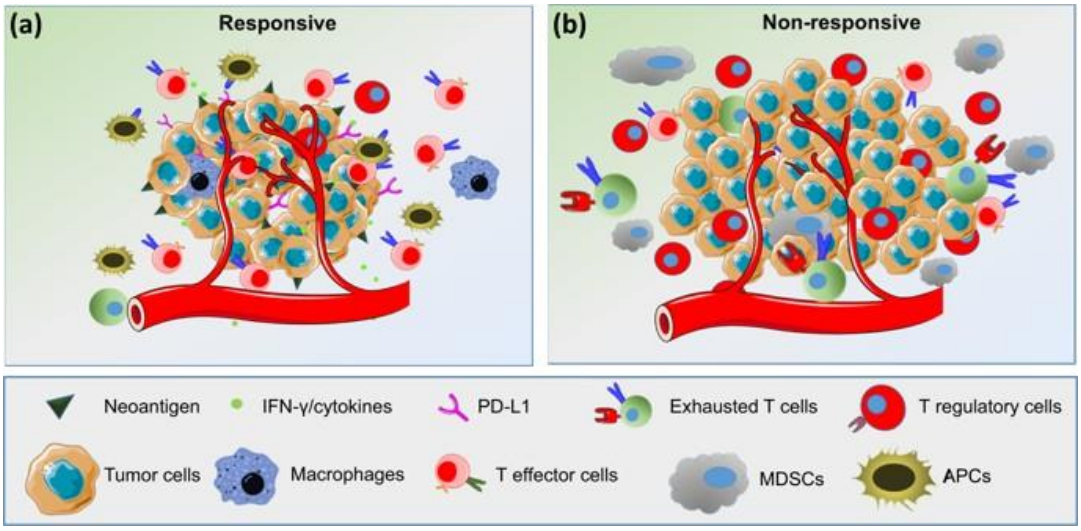

Figure 5. A diagram showing responsive “hot” tumours versus non-responsive “cold” tumours. Adapted from [23].

Hot tumours often have a high mutational load, therefore have higher levels of neoantigens that can be recognized by the immune system. Tumour-infiltrating T cells are lymphocytes in the immune system that accumulate in the TME and play a crucial role in the body’s fight against cancer. There are multiple types of T cells in the body.
CD8+ T cells identify the cancer antigen and directly kill it after understanding cues such as development linage [23]. CD4+ is another type of T cell which carries out a number of immune responses which either enhances or weakens the immunity against the cancer [23]. Both these T cells have been found in a wide variety of cancers such as lung cancer, breast cancer, bladder cancer etc. These immune cells are actively identifying and destroying cancer cells, but because the tumour employs a variety of immunosuppressive strategies, it may still avoid total elimination. Because the immune system is already actively involved in the battle against the cancer cells, hot tumours are frequently more receptive to immunotherapy.

The immune response to the cancer cells in cold tumours is weak or nonexistent. Cold tumours frequently have an immunological milieu that discourages the activation of immune cells. The TME of cold tumours have high levels of immunosuppressive cells, such as Tregs and MDSCs, and very low levels of NK cells (CD8+) and activated lymphocytes [23]. Because there are few immune cells accessible to target the cancer cells, they are thus less responsive to immunotherapy treatments.

III. IMMUNOTHERAPY

Comparing Immunotherapy to Conventional Treatments

Immunotherapy is an approach to cancer therapy that helps support the immune system to fight cancer. Your immune system assists your body in fighting infections and other diseases[41]. Complex systems have been developed by the immune system to discern between healthy cells and harmful invaders. However, by using these pathways, cancer cells frequently manage to avoid detection. They can create defences against immunological attack or reduce the immune response[41]. With the help of immunotherapy, the immune system will be better able to identify and combat cancer cells by thwarting these evasive tactics. White blood cells, organs, and lymphatic system components make up the composition of the immune system. Some therapeutic strategies include Immune checkpoint inhibitors, anti-CTLA-4 and anti-PD-1 inhibitors, T-cell transfer therapy, Monoclonal Antibodies etc[41]. These strategies are most effective when deployed in the equilibrium phase of the tumour.

Immunotherapies have advantages when compared to conventional treatments such as surgery, chemotherapy, radiation therapy, targeted therapy and hormone therapy. Even though conventional treatments have evolved over time with improved survival rates and better outcomes they have serious side effects. The side effects for conventional treatment ranges from nausea, vomiting and alopecia in chemotherapy; skin changes, long term risks of secondary cancers, and swelling and fluid buildup in radiation therapy; and in surgery there could be scarring and discomfort around the surgical site and constant risk of bleeding[42]. Immunotherapy on the other hand follows a targeted approach. The nature of the immune system is highly specific which means that it only targets the area of the tumour and not the healthy tissues in the body around the tumour. This is not the case with conventional treatment which targets healthy tissues in the body as well. As a result, immunotherapies have less severe side effects compared to conventional treatment, for example in most immunotherapies the patient will only exhibit flu symptoms like mild-fever, and rashes that can be easily managed[41].

Immunotherapies have also been proven, through certain cases, to be more effective than traditional methods in improving both progression-free survival and overall survival as represented in figure 6 which shows the advantages of pembrolizumab, a highly selective, humanized antibody against PD-1 over traditional chemotherapy in patients with non-small-cell lung carcinoma [24]. However, there are still many patients who do not respond to immunotherapy and likely have different tumour-immune dynamics that are individual to the patient. Highlighting the requirement for continued research in this field.

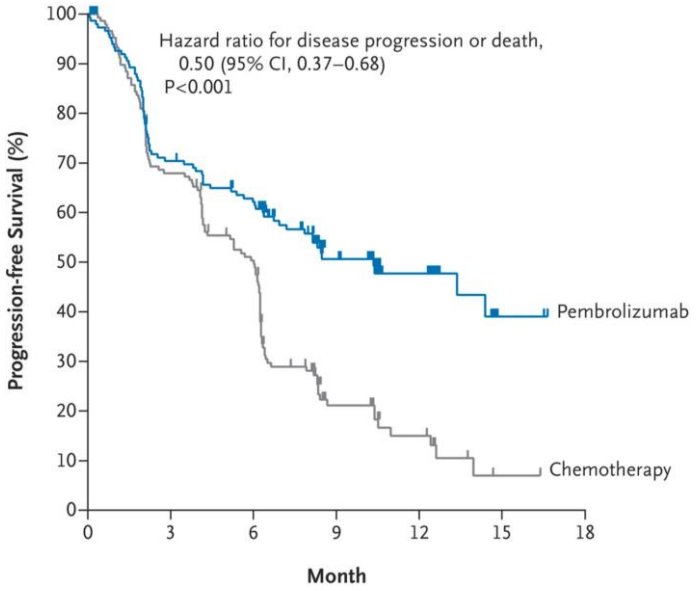

Figure 6. Kaplan Meier curve showing a comparison of progression-free survival when treated withchemotherapy or pembrolizumab in patients with NSCLC. Adapted from [24].

A major advantage of immunotherapy over conventional methods is that immunotherapies offer long lasting effects of cancer remission. Chemotherapy tends to target the metastasis of cancer to a secondary organ and are only effective during the course of treatment. In contrast, immunotherapies offer the possibility of long-term protection from cancer. An example of this is due to a phenomenon known as epitope spreading whereby the antigen targeted is spread through the immune system via the adaptive immune system, similar to the generation of antibodies against a virus or

bacterium. This leads to the development of an immune response against further cancer-causing epitopes (25). Additionally, this introduces a cross-generational inheritance of an immune response to cancer. For example, the inheritance of certain genes or mutations can predispose one to cancer such as mutations in the BRCA gene and its link to breast cancer (26). Therefore if you have long-term immunity to cancer you can circumvent the effects of cancer as a result of genetic inheritance.

Together, the advantages of immunotherapies over conventional therapies offer better outcomes and lower side effects leading to patients obtaining a better quality of life.

Emerging Therapies

Emerging therapies in immunotherapy represent a promising frontier in the battle against various diseases, particularly cancer. Among these cutting-edge approaches, antibody therapy, checkpoint inhibitors, and adoptive cell transfer are gaining increasing attention. These innovative strategies are revolutionising the field of immunotherapy, offering new avenues for personalised and highly targeted treatments with the potential to transform the way we approach and treat various medical conditions.

Monoclonal antibodies

The use of monoclonal antibodies in cancer treatment has proven to be a useful and cutting-edge strategy. Utilising monoclonal antibodies, this type of targeted therapy specifically targets proteins or other molecules found on the surface of cancer cells or in the environment around them. Some examples of monoclonal antibodies are trastuzumab and rituximab[43]. In order to achieve the best results, monoclonal antibody therapy for cancer is frequently used in conjunction with other therapies like chemotherapy or radiation therapy, and researchers are always looking for novel targets and treatment options. With more precision, fewer side effects than conventional treatments, and better overall results, these therapies have transformed the therapy landscape for many patients[43]. Although not all patients will have the same benefits, the response to monoclonal antibody therapy can differ between individuals and disease types.

Immune Checkpoint Inhibitors

Immune checkpoints are proteins that are essential for controlling the immune system and preventing it from attacking healthy cells. Cancer cells, however, can manipulate these checkpoints to avoid being recognised by the immune system[44]. By disabling these checkpoints, checkpoint inhibitors enable the immune system to more easily recognise and combat cancer cells by "releasing the brakes" on it. There are two main checkpoint inhibitors used: anti-PD-1 and anti-CTLA-4. These medications have had a great deal of clinical success and have changed how some patients with metastatic or advanced malignancies are treated[44]. Therefore, Immunotherapies such as checkpoint inhibitors anti-CTLA-4 and anti-PD-1 inhibitors are a step forward to treat patients suffering from this gruesome disease. It will decrease the death toll, increasing the quality of life around the world.

Adoptive Cell Transfer

The advanced cancer immunotherapy technique known as adoptive cell transfer (ACT) involves the removal, alteration (if required), and reinfusion of a patient's immune cells, mainly T cells, in order to more precisely target and eliminate cancer cells. Adoptive T Cell Transfer Therapy enhances the innate capacity of T cells to combat cancer by bolstering the population of immune cells capable of targeting cancerous cells within the body. T cells possess the ability to recognize foreign antigens situated on the surface of pathogens and subsequently engage with these foreign substances to elicit an immune response. This therapeutic approach involves the extraction of T cells from the patient's body, which can be sourced either from the tumor site or from the bloodstream. Following this, conventional treatment methods are applied to patients in order to enhance the likelihood of robust T cell growth and activation once they are reintroduced into the body. This process effectively supports the utilization of Adoptive Cell Transfer Therapy (27). The delivery of this immunotherapeutic approach to the patient is accomplished through an infusion, with the primary objective of restraining the proliferation and metastasis of tumor cells, thereby controlling their spread (28). Clinical outcomes of Adoptive Cell Transfer therapy with tumor-infiltrating T cells have demonstrated clinical responses in a range of 50% to 72% of patients, including complete responses in 10% to 40% of cases (29).

When it comes to treating specific types of cancer, especially those that have shown resistance to previous therapies, ACT is a highly personalised approach that has achieved extraordinary results. A more advanced immunotherapy based on ACT is chimeric antigen receptors (CARs)-T cell therapy. CARs are engineered, artificial receptors that combine, in a single chimeric protein, the recognition domain of a specific antibody

against a tumour-associated antigen and an intracellular signalling domain capable of activating T cells [30]. Immune cells are removed from the patient, like all ACT methods, however the T cells are then genetically engineered to express CARs with a specific antigen (figure 7) before being injected back into the patient. Gene editing of T cells has previously been done with techniques such as TALEN or zinc finger nucleases however more recently CRISPR/Cas9 is being implemented.

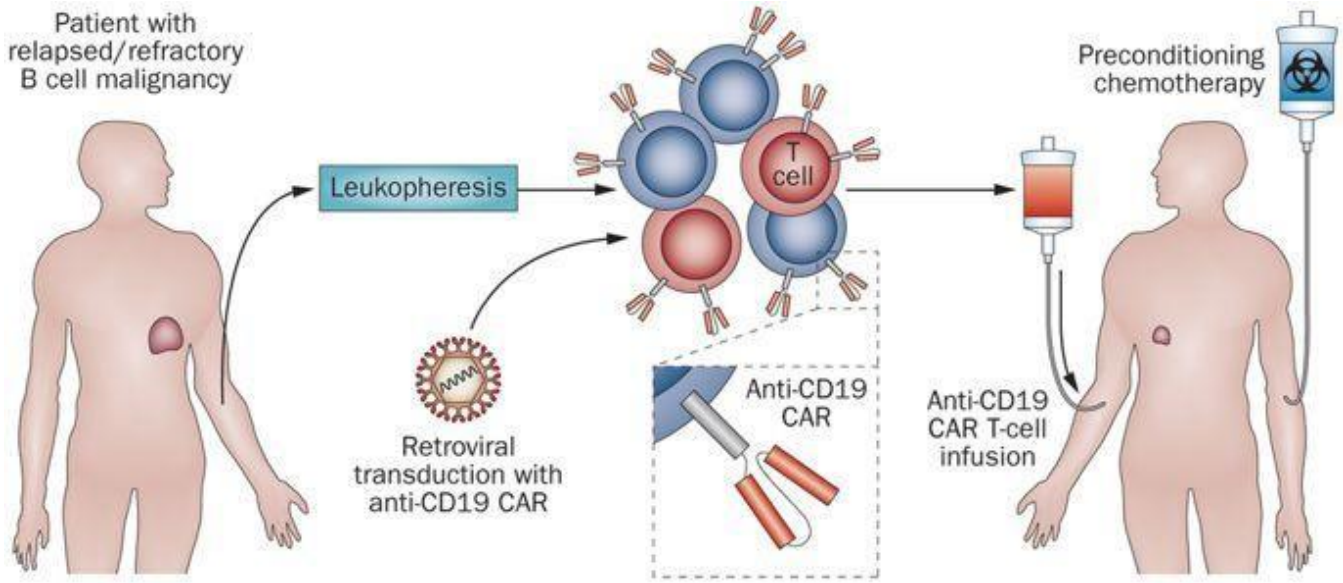

Figure 7. A schematic showing adoptive T cell transfer with engineered CAR-T cells targeting CD19 in a patient with relapsed/refractory B cell lymphoma. Adapted from [30].

The final CAR-T cells operate to redirect lymphocytes to recognise and kill cells displaying a particular target antigen. CARs have four main structural components: (1) a single-chain variable fragment (scFv) of an antibody, which provides target specificity (2) a hinge and transmembrane region (3) a co-stimulatory domain (4) and a T-cell-activation domain [31]. CAR-T cells recognise and bind with ScFv region tumour cells. The binding causes intercellular signalling in CAR-T cells which leads to activation of CAR-T cells and rapid proliferation. The CAR-T cells can then attack and kill cancer cells.

However, there can be a CAR-T cell failure for multiple reasons. First, CAR-T cell failures have several causes: for some patients, the CAR-T cell product cannot be successfully manufactured or the generated CAR-T cells do not expand sufficiently (either during manufacturing in vitro or after administration in vivo) [32]. Second, antigen modulation by the cancer themselves can enable antigen escape as a mechanism of resistance [32]. Third, the characteristic toxicities of CAR-T cell therapy such as severe cytokine-release syndrome (CRS) and/or neurotoxicity. These toxicities can be fatal and remove the potential for therapeutic benefit in a small proportion of patients [32]. Furthermore, there are likely additional and unknown cytotoxic effects.

IV. MRNA VACCINES

A new therapeutic strategy called mRNA vaccines have also gained more popularity lately. Unlike traditional vaccines, which use dead or weakened viruses, these vaccines use mRNA with the instructions for making a cancer antigen[33]. When it's injected, this mRNA guides some of our cells to make harmless antigens, stimulating the immune response to attack cancer cells. The cells produce a targeted pathogen (usually protein). This protein subsequently sets off an immunological response that results in the development of antibodies. Due to their quick creation and effective application in the fight against infectious diseases, most notably during the COVID-19 pandemic, mRNA vaccines have drawn a lot of attention and are beginning to be applied to other areas of disease such as cancer.

Mechanism of Action

Innate immune sensing of mRNA vaccines is an important aspect of how these vaccines work. As shown in figure 8 there are two types of immune sensing, one is of unmodified, unpurified mRNA and the other is of nucleoside-modified, purified mRNA. Immune sensing of unpurified mRNA is a complex process as the immune system responds to the presence of foreign RNA molecules that haven't been modified or purified in the way that mRNA vaccines are generally developed[34]. Purified mRNA on the other hand follows an opposite approach in which purified antigens or immunogens delivered into the body are detected by the immune system, which then

reacts. Purified antigens are separated, clearly defined elements of a disease or other foreign substance that are utilised to deliberately and carefully activate the immune system[34].

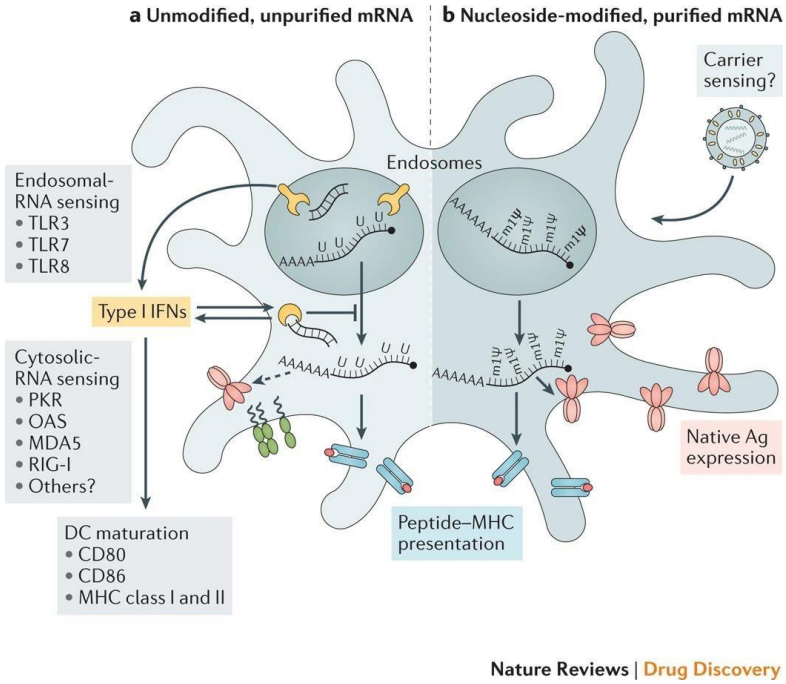

Figure 8. The figure shows the innate immune sensing of mRNA vaccines. Adapted from [34].

Types of mRNA Vaccines

There are two main types of mRNA vaccines which are used to treat cancer: the first is non-replicating mRNA which encodes the target antigen. A tiny fragment of artificial mRNA that codes for a modified antigen against the cancer, called cancer epitopes is present in the vaccination[35]. The mRNA is injected into the patient's cells during the vaccination process, typically in the muscle tissue. Ribosomes in the cells use the mRNA to translate it into the cancer antigen. However, the antigen is modified to the extent that the immune system mounts an immunological response against it after identifying it as alien. T-cell activation and antibody synthesis are both involved in this. The immune system "remembers" the antigen after the immunological reaction has been set off. As a result, memory cells are created, which will re-activate should the cancer return.

The second type of mRNA vaccine is virally derived, self-amplifying RNA (saRNA). Similarly to mRNA vaccines, SaRNA vaccines include synthetic genetic material, however, the genetic material in saRNA vaccines contains extra components required for replication and amplification in addition to the instructions for making the target viral protein. Usually through an injection, the saRNA is given to the recipient and transported to their cells.

The additional replicative ability of saRNA vaccines allows for replicating within the cells, leading to abundant antigen production, thus inducing a more severe immune response [36]. The replicative components are recognised by the protein synthesis machinery of the cells. The target protein that results from the translation of the saRNA can act as a template for the creation of more saRNA molecules. An immune response that is more powerful can be produced by the multiplication and amplification of saRNA within the cells. The combination of saRNA molecules and the elevated target viral protein production could result in a more sustained immune response and longer-lasting immunity.

Application of mRNA Vaccines in Cancer

The genetic make-up of each patient's tumour makes cancer a highly varied disease. Traditional cancer treatments are frequently broad-based and may be unable to successfully target certain mutations. Highly individualised cancer treatment plans can now be developed thanks to the discovery of patient-specific cancer epitopes, or mutanomes. For example, a recent paper published a protocol for identification of patient-specific cancer epitopes or ‘mutanome’ isolated heterogeneous populations of cancer cells, sequenced them and used a machine learning model to identify potential mRNA vaccine targets, whereupon they generated an mRNA vaccine [37].

The process of genomic sequencing begins by researchers isolating cancer cells from a diverse population of cancer cells after which they sequence and start the procedure. The genetic alterations present in a tumour can now be

precisely and thoroughly characterised because of developments in genomic sequencing technologies. This makes it possible to pinpoint the precise mutations that fuel the development of cancer. In this strategy, machine learning is essential. Machine learning techniques are able to locate prospective mRNA vaccine targets by examining the enormous amount of genetic data produced. These models are able to foretell which mutations will most likely elicit an immunological response and which ones will be good candidates for vaccine development. This is an outstanding illustration of a multi-disciplinary approach to oncology treatment and the potential to transform healthcare. Researchers can then create patient-specific mRNA vaccines once they have identified possible vaccine targets[34].

The goal of these vaccinations is to activate the patient's immune system so that it can recognise and target cancer cells selectively. The mRNA technology enables the quick production and customization of vaccinations specific to the individual mutanome of each patient. Compared to conventional cancer therapies like chemotherapy and radiation therapy, patient-specific mRNA vaccines have the potential to be far more successful and have fewer adverse effects. They only affect cancer cells while sparing healthy tissue which is uncommon in conventional treatments[34].

Limitations and Drawbacks

Even though mRNA vaccines are considered safe due to their short manufacturing period the chances of contaminants. Among the potential negative consequences of non-native nucleotides and delivery system components are local and systemic responses, inflammation, biodistribution, induction of auto-reactive antibodies, and local and systemic reactions[34]. Other things to keep in mind during the next clinical and pre-clinical studies would be the potential risk of non-native nucleotides and delivery system components[34]. Therefore, as various mRNA methods and delivery systems are used for the first time in people and are tested in larger patient groups, safety will need to be continually evaluated. Once the side effects are dealt with, patient specific mRNA vaccines could be a game changer in finding a cure to cancer, however, it raises serious ethical concerns.

Despite extensive research being done in the field of mRNA vaccines there are a lot of ethical considerations which need to be kept in mind before these vaccines are commercialised. First would be the exorbitant cost of these vaccines which makes it available to a very small fraction of the population. This would create a population super rich and super healthy putting the less wealthy at a disadvantage. This promotes a mindset of discrimination between the rich and the poor and also promotes the idea that the key to getting better healthcare is always money. This would also cause the rich to be prone to less health diseases which can result in a domino effect to great disaster.

IV. DISCUSSION

The immune system is crucial in the fight against cancer since cancer cells can only live and spread by evading this defence mechanism. Tumour cells are shielded from the immune system's defences by preventing immunological activation, especially T-cell activation, which is crucial. The immune system can also be subverted, suppressed, or exploited by tumour cells in response to it, improving the conditions necessary for tumour cells to flourish and continue to develop and proliferate. However, the introduction of immunotherapies has allowed humans to lessen the cancer cells' evasion strategies.

Immunotherapies have helped to strengthen the immune system's ability to combat cancer and have produced more effective results than traditional forms of treatment. They have caused the clearance of tumours and immune escape, which has reduced the influence of immunosuppressive mechanisms expressed by tumours and constrained tumour growth. The method we currently treat cancer has been transformed by immunotherapies, which also have long-term cancer protection. However, new immunotherapies must still be created in order to improve immunotherapies as a therapy option for patients who have not responded to earlier immunotherapies.

Despite the fact that our knowledge of the interactions between tumours and the immune system is still developing and there is still much to learn about this disease that has had a terrible impact on the world's population, we have seen significant advancements in immunotherapies and the way that cancer is generally treated. Immunotherapies constitute one tool in the ever-expanding toolbox of cancer medicines because they are likely to not be appropriate for everyone.

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