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Exploring New Strategies to Overcome Immunotherapy Resistance in Non-Small Cell Lung Cancer

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ABSTRACT

Non-small cell lung cancer (NSCLC) is a disease that has been negatively impacting the lives of tens of thousands of families each year. Yet, despite consistent research efforts, a cure for this cancer still hasn't been developed. Though there are many treatment options for NSCLC, the majority of these are not completely effective and tend to have numerous side effects that come with them. Over the past few decades, immune checkpoint inhibitors (ICIs), a common form of immunotherapy, have risen as a promising discovery in cancer treatment research. Unlike other treatment types, ICIs work to activate the immune system's natural response to antigens by blocking checkpoint proteins which have the ability to inhibit immune cell function. Particularly for NSCLC, ICI treatment has shown many encouraging outcomes, due to its ability to work well against the complexity of the disease. However, there's only one downside: many NSCLC patients tend to develop either primary or acquired resistance to treatment, due to numerous factors involving genetic mutations that are unique to the tumors in each individual. This paper primarily focuses on identifying the most common NSCLC treatment resistance mechanisms, and exploring the most effective methods involving ICIs in order to overcome this resistance.

Introduction

Worldwide, lung cancer is the biggest contributor to cancer mortality. A large portion of this is due to advanced stage diagnosis, which causes patients to have limited treatment options. Immune checkpoints are proteins that help to regulate T cell function. However, sometimes these checkpoints can prevent T cells from identifying cancerous cells as threats in the immune system. Immune checkpoint inhibitors or ICIs, a form of immunotherapy, work to inhibit immune checkpoints, including CTLA-4, PD-L1, or PD-1, in order to boost immune activation. Though the use of ICIs have shown effectiveness for some cancer types, many NSCLC patients either do not respond or quickly develop resistance to these treatments. In order to overcome this, scientists have started to investigate possible alternative checkpoint molecules that can be potentially targeted with new ICIs to enhance T cell response against cancer, Researchers have also begun looking into treatments which combine immunotherapy with other therapies, including radiation and chemotherapy. Combination therapies can allow multiple aspects of the tumor to be attacked at once, making it more difficult for the tumor to resist the treatment. Additionally, by utilizing inhibitors to block multiple immune checkpoints simultaneously, researchers can overcome the immunotherapy resistance that many patients have been dealing with and create an improved anti-tumor immune response in the body.

Around the world, immunotherapy appears to be one of the most rising new cancer treatments. This treatment approach has the ability to revitalize the immune system's ability to recognize and eradicate tumors. Scientists in multiple different countries have begun to research new methods of immunotherapy and many have even been successful at treating advanced lung and melanoma skin cancers. In a study conducted at Peking Medical College Hospital, China, 97 NSCLC patients received nivolumab or pembrolizumab, ICI treatments which worked to block the PD-

1 and PD-L1 proteins (Chen et al., 2020). However, only 16.5% patients responded to the treatment and 60.8% maintained the stable disease; this is a relatively low percentage compared to the success rates seen in other cancer types. The contributing factors to the low success rates could include a lack of combination therapy, which would reduce the chance of successfully targeting the antibodies, or prior treatment history, which could've allowed patients to develop resistance to the ICIs during treatments taken prior to the study.

In the United States, the usage of immunotherapy to treat lung cancer has rapidly grown. Though many NSCLC patients have experienced long term benefits through ICI treatment, numerous patients tend to have disease progression during or after treatment ends, due to development of either primary or adaptive resistance to the therapy. Many studies are being conducted to address this issue. Recent research has shed light to many new immune checkpoints, including TIGIT, VISTA, B7-H6, NKG2A, TIM-3, Galectin 3, LAG-3, and more. These targets have been studied intensely in various studies and have shown promising outcomes (Patwekar et al., 2024).

One of the most prominent medical institutions in the United States, Johns Hopkins Medicine, conducted a trial comparing (anti PD-L1 immunotherapy agent) and the combination of durvalumab with one of three other forms of immunotherapy as treatment for early-stage NSCLC. The response rate was much higher in patients receiving a combination treatment. According to the researchers, the results from the trial indicate that simultaneously targeting multiple cancer pathways may be preferred to utilizing a monotherapy (Cascone et al., 2023).

Even within the U.S, the world's leading country in cancer research and treatment, a patient dies of lung cancer every four minutes (*Lung Cancer Key Findings*, 2023). By understanding resistance mechanisms in immunotherapy and developing new therapies to mitigate it, researchers can improve treatment outcomes and ultimately increase the chances of survival for lung cancer patients around the world. The goal of this literature review is to establish the current understanding of ICI treatment for NSCLC, understand resistance mechanisms in this treatment, and explore potential methods being investigated to overcome this resistance.

Methodology

This paper's primary goal is to examine the immunotherapy's current status for NSCLC and analyze upcoming strategies to better improve treatment and overcome resistance mechanisms. This research was conducted through a comprehensive secondary literature review based on numerous informational articles, studies, and trials. The research process began with an analysis of various articles which provided a brief overview of the current status of traditional immunotherapy, particularly immune checkpoint inhibitors (ICIs) for NSCLC. Though this treatment showed many highly successful results, a recurring concern being presented was the resistance to these ICIs, found in the immune systems of many NSCLC patients. Further exploration indicated that the source of the issue was the tumor's adaptations to treatments over time, allowing it to eventually resist the ICIs targeting of typical checkpoints to a certain extent. Analysis of various clinical trials indicated that NSCLC patients receiving treatment through combination ICI therapies incorporating other immunotherapy agents or other kinds of therapies, were less likely to develop treatment resistance, as opposed to patients receiving the traditional ICI treatment as a monotherapy. It is crucial to expand the concept of immunotherapy by developing more effective and successful cancer treatments that can successfully treat a broader range of patients. This secondary literature review was conducted completely through the usage of online resources from researchers across the globe, in order to mitigate any potential biases and receive a complete understanding of the concept. No physical materials or resources were used to conduct the research.

An Overview of Non-Small Cell Lung Cancer

As of now, the likelihood that an adult in the USA will develop lung cancer is 1 in 16 (*Key Statistics for Lung Cancer*, 2024). Despite its prevalence, there is still no completely effective treatment for this cancer. The most common therapies for lung cancer include chemotherapy, surgery, immunotherapy, and radiation therapy. More recent treatments,

including stereotactic radiation therapy, targeted therapy, and newer forms of immunotherapy, are also being studied and developed.

Process of Lung Cancer Development

All cells in the human body contain DNA, which provide them with a set of instructions that they need to multiply, grow, and self-destruct at a set rate. However, in cancerous cells, this DNA can instruct the cells to multiply more quickly and indefinitely, without undergoing apoptosis, a form of self-destruction or programmed cell death. This can cause the cancer cells in the body to rapidly grow in a certain body part. These cancer cells might eventually form a tumor, a mass which can grow and invade healthy organs and tissue. Over time, cancer can become metastatic, or spread to other areas in the body. The two types of lung cancer are non-small cell (NSCLC) and small cell (SCLC), which are primarily distinguished based on how the tumorous cells look when viewed underneath a microscope. NSCLC is a broad category that contains various subtypes of lung cancers, the most common of which are adenocarcinoma, large cell carcinoma, and squamous cell carcinoma. Though smoking is said to be the greatest cause of lung cancer, non-smokers also have the ability to develop NSCLC through risk factors including secondhand smoke, having lung cancer in family history, and long-term exposure to radon. Alternatively, SCLC only represents less than 20% of lung cancer diagnoses and typically only occurs in people who are or have been long-term smokers (Ciupka, 2020).

Current Treatment Options

Though NSCLC makes up almost 9 out of every 10 lung cancer diagnoses, it can be much more difficult to treat than SCLC; a non-small cell tumor spreads at a fairly slower rate, reducing the chances of being able to diagnose the cancer at an early point (*Key Statistics for Lung Cancer*, 2024). Though early-stage detection and diagnosis has been improving, less than 25% of NSCLC patients are diagnosed before stage III, leading to lower survival rates. Researchers across the globe are working to advance our current understanding of lung cancer, specifically NSCLC, and its treatments. Early-stage NSCLC, stages 0 and I, can be treated with a surgical removal, which can involve a segmentectomy, wedge resection, or lobotomy. Following surgery, adjuvant treatments, including Osimertinib (a targeted therapy used to treat patients with an abnormal EGFR, epidermal growth factor receptor, that can allow the cancer to metastasize), atezolizumab and pembrolizumab (immunotherapy drugs), and chemotherapy drugs, can be used in order to prevent recurrence of the cancer. Stage II NSCLC may involve neoadjuvant (prior to surgery) chemotherapy while stage IIIA will typically involve chemotherapy, radiation therapy, and/or surgery. Stage IIIB NSCLC indicates that the tumor may have spread to lymph nodes close to the neck, other lobe of the lung, or in the chest. These cancers can be treated through radiation, chemoradiation, or possibly immunotherapy. At the IVA or IVB stage, treatments vary depending on the cancer's gene or protein changes and the individual's overall health (*Treatment Choices for Non-Small Cell Lung Cancer, by Stage*, 2024). Researchers are working on developing more effective treatments to better combat the advanced stages of NSCLC, including more recent forms of immunotherapy and targeted therapy.

Immunotherapy as a Cancer Treatment

The most common therapies utilized to treat NSCLC are chemotherapy and radiation therapy. These therapies are not completely successful and come with many side effects. Chemotherapy involves the use of drugs that contain powerful chemicals which kill rapidly growing cells in the body. However, while destroying malignant cells, these drugs often kill many healthy cells as well. Radiation therapy applies intense radioactive energy in order to destroy cancer cells. Similar to chemotherapy, this treatment can damage healthy cells located near cancer cells in the body. Many researchers have turned to immunotherapy and other forms of treatment, like targeted therapy, to prevent healthy cells from being damaged, while still effectively eradicating tumorous cells.

Fighting Cancer Cells Within the Immune System

T-lymphocytes (T cells) are a crucial type of white blood cell that have a major role in the immune response. There are two main types of T cells: CD4+ T cells (T-helper cells) and CD8+ T cells (cytotoxic-T cells). While CD4+ T cells activate other immune cells, CD8+ T cells have the ability to directly destroy cancerous cells. When a cancer cell dies, it releases proteins which are then taken in by antigen-presenting cells (APCs). APCs work to split up these proteins into peptide fragments (antigens) and bind them to major histocompatibility complex (MHC) molecules found on their surface, in order to present them to T cells. Through interactions between the T cell receptor (TCR) and the MHC-peptide complex, the T cells are 'trained' to effectively recognize the peptides on malignant cells and destroy them. There are three common MHC molecules, class I, class II, and class III proteins, which can be differentiated based on their genetic variability; only class I and class II are involved with antigen presentation to T cells. CD4+ T cells can identify peptides bonded with MHC class II molecules and CD8+ T cells can identify peptides bonded with MHC class I molecules. Once the foreign cancer antigens are identified, CD4+ T cells work to coordinate the immune system and activate other immune cells including macrophages, B cells, and CD8+ cells. On the other hand, once CD8+ cells have been activated, they can directly produce and release cytokines and enzymes called perforins, in order to kill the malignant cells that display the same peptides on their surface (Sauls et al., 2023).

Advancements in the Field

Over time, tumorous cells have developed numerous escape mechanisms against the immune response. One method is checkpoint molecule expression, such as PD-L1, which can prevent T cells from attacking malignant cells. Malignant cells can also form an immunosuppressive tumor microenvironment (TME) by producing cytokines, including TGF-β (transforming growth factor beta) and IL-10, in order to weaken T cell function. Additionally, cancer cells can develop resistance to apoptosis. Other mechanisms that aren't present as often in lung cancer cells include MHC class I downregulation and antigen masking.

Immunotherapy is a groundbreaking treatment in the cancer field which activates the immune system's natural defense methods in order to resist these immune-evading mechanisms presented by tumorous cells. Currently, there are four types of immunotherapy treatments being used or tested for lung cancer: immune checkpoint inhibitors, monoclonal antibodies targeting growth factor (tyrosine kinase) or angiogenic receptors, cancer vaccines, and adoptive cell therapy (Table 1). As of now, the FDA has approved several checkpoint inhibitors and monoclonal antibodies to treat lung cancer, while other treatment types are still being tested and further developed in clinical trials. In some cases, using immunotherapy as a combination therapy has also been FDA approved. Immunotherapy works to boost the immune response against malignant cells. It does this by blocking specific proteins which aid cancer cells from evading immune cells that are trying to identify and attack them. Depending on the form of immunotherapy being used, it may be given orally, through a vein, or through the bladder (Boyce, 2024).

Primary Types of Immunotherapy Treatment and their Functions

Treatment type	Functions	Examples (in various types of cancer)
Immune Checkpoint Inhibitors (ICIs)	ICIs block checkpoints, which are exploited by tumorous cells, in order to allow T cells to effectively recognize and attack tumors.	atezolizumab (Tecentriq) pembrolizumab (Keytruda) ipilimumab (Yervoy)

Monoclonal Antibodies (mAbs)	mAbs work to directly target malignant cells by binding to specific antigens and attempting to destroy them through various mechanisms.	rituximab (Mabthera) trastuzumab (Herceptin) cetuximab (Erbitux)
Cancer Vaccines	Vaccines are composed of tumor antigens/cells or modified products in order to stimulate the immune system and keep it prepared for a real attack.	Bacillus Calmette-Guerin (BCG) Vaccine Sipuleucel-T Vaccine
Adoptive Cell Therapy (ACT)	ACT works to modify and enhance an individual's immune cells in order to improve immune response to malignant cells.	Tumor-Infiltrating Lymphocytes CAR T Cell Therapy (TIL) Therapy

Source: Thatikonda, 2024

Researchers worldwide view immunotherapy as a pioneering breakthrough in lung cancer treatment, with numerous clinical trials and reviews being conducted to evaluate and improve its efficacy. A systematic review of seven different randomized clinical trials investigated the combination of erlotinib (a tyrosine kinase inhibitor that prevents cancer cell growth) and multiple mAbs compared to erlotinib alone, as treatment for patients diagnosed with advanced non-small cell lung carcinoma. The data showed a 40% decrease in the hazard ratio (HR) or risk of death, as well as a major improvement in the progression-free survival (PFS) rate for the combination therapy. Compared to the monotherapy, the combination showed very little improvement in the overall survival (OS) rate, and the researchers concluded that the value was not high enough to be considered a statistically significant finding. Additionally, there was a relatively lower amount of severe treatment-related adverse events (AEs) in the erlotinib monotherapy group (Liu et al., 2023). Though the study shows the many potential benefits of the combination therapy over the monotherapy, it also highlights the drawbacks, including the minimal improvement in OS and the considerable increase in side effects; this indicates that further research is needed before the combination of the mAbs and erlotinib can be seriously considered as a possible treatment.

Usage of Immune Checkpoint Inhibitors (ICIs)

Often, immune checkpoint proteins can use their ligands to bind to partner proteins found on malignant cells, causing them to transmit false signals that tell immune cells to deactivate their immune response. Shutting off the immune response inhibits T cells from effectively identifying and attacking cancerous cells. Immune checkpoint inhibitors (ICIs) are used to block the binding that takes place between immune and tumorous cells (Figure 1). ICIs are one of the most common forms of immunotherapy, due to their ability to treat a broad variety of tumors while maintaining consistent responses. As of now, there are six FDA-approved ICI agents for NSCLC targeting PD-1, PD-L1, or CTLA-4 (Olivares-Hernández et al., 2023), as shown in Table 2 below.

Current Proteins Targeted by FDA-Approved ICI Agents for Lung Cancer

Targeted Protein	Current ICI Agents	Cells Expressing	Mechanisms of Immune

		Target	Response Evasion
Programmed Cell Death Protein 1 (PD-1)	Pembrolizumab Cemiplimab Nivolumab	B cells CD4+ T Cells Natural Killer (NK) Cells CD8+ T Cells	Tumors can express PD-L1, which binds to PD-1 on T cells, preventing cytokine production and inhibiting T cell activation that allows tumors to be effectively identified and attacked.
Programmed Cell Death Ligand 1 (PD-L1)	Durvalumab Atezolizumab	Malignant Cells Antigen-Presenting Cells (APCs) Some Immune Cells	Blocking PD-L1 can prevent tumors from interacting with T cells that express PD-1, allowing these T cells to more effectively function and destroy tumorous cells.
Cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4)	Ipilimumab	CD4+ T cells T regulatory cells (Tregs) CD8+ T cells	CTLA-4 competes with CD28, a costimulatory receptor, to bind to B7 on APCs. Blocking CTLA-4 can reduce competitive inhibition of T cell activation.

Source: Thatikonda, 2024

In an international study conducted across 13 countries, scientists compared the effectiveness of atezolizumab (ICI) and docetaxel (chemotherapy), as treatment for previously treated NSCLC patients (Fehrenbacher et al., 2016). The data from the randomized trials indicated that the median OS was significantly higher by 2.9 months for the atezolizumab patients. Additionally, results showed that the docetaxel group had a 27% increase in death risk and a 28% increase in the percentage of patients who were experiencing drug-related AEs. In a similar study comparing the efficacy of docetaxel to a different ICI, nivolumab, patients with advanced non-squamous-cell lung carcinoma were thoroughly studied (Paz-Ares, 2015). Similar to the previous study, the results indicated that the patients receiving the docetaxel had a 27% increase in death risk and a 43.2% increase in grade III or higher AEs. The nivolumab group demonstrated a superior OS by 2.8 months. In both cases, the ICIs significantly improved survival rates while still resulting in a lower amount of treatment-related side effects. The atezolizumab and the nivolumab both showed to be tolerable and effective treatments than the docetaxel for NSCLC patients.

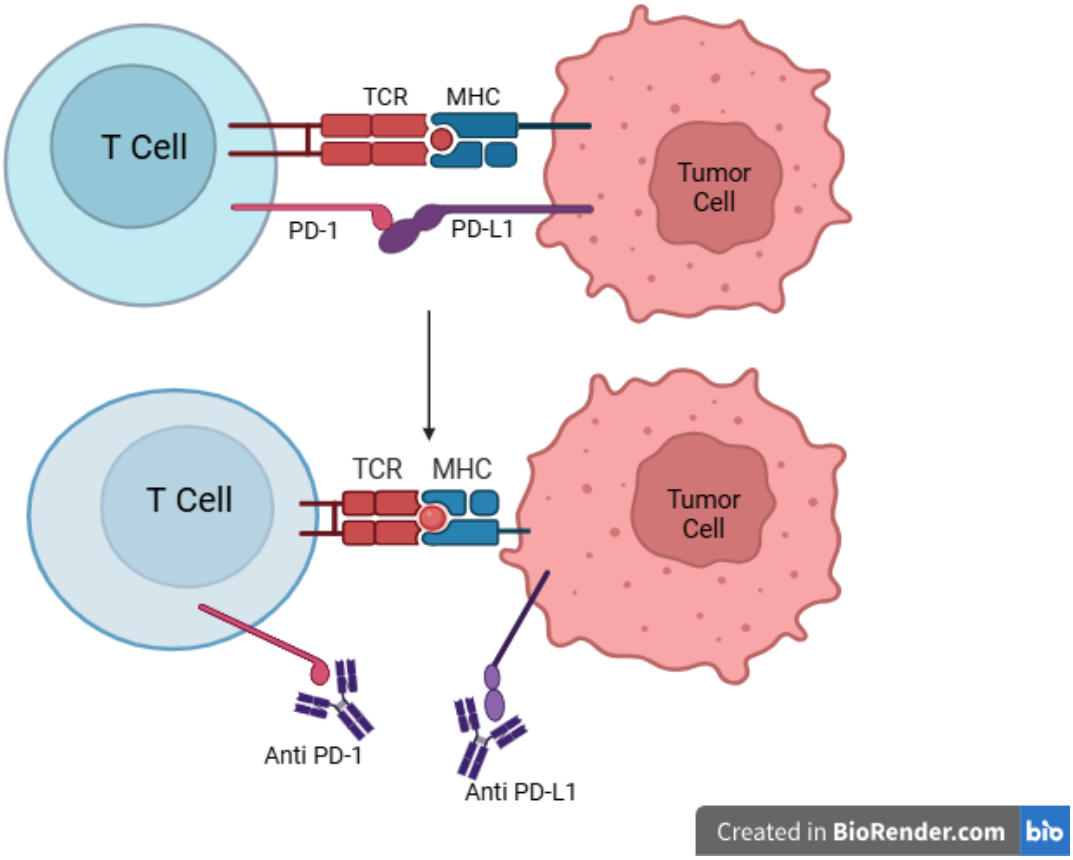

Figure 1. How ICI treatments (Anti PD-1 and Anti PD-L1) work to block checkpoint proteins from binding T cells and tumor cells and inhibiting T cell function. Source: Thatikonda (created using BioRender.com), 2024

Mechanisms of Resistance to Treatment

Despite its promising aspects, immunotherapy can come with various complications, especially in lung cancer. The complexity of NSCLC often makes patients more likely to develop resistance to immunotherapies. Resistance can be categorized into two broad categories, primary and acquired resistance. Primary resistance refers to when a patient's tumor doesn't respond to a treatment at all throughout the treatment duration. Contrariwise, acquired resistance refers to when a patient has an initial response to the treatment, but eventually develops disease progression over time. There are numerous mechanisms which assist tumors in resisting immunotherapy treatment and these tend to vary depending on an individual's conditions. In NSCLC, the most common resistance mechanisms include weak immunogenicity, defective antigen presentation, and abnormal signaling pathways.

Immunogenicity is the ability of a foreign substance or cancer to provoke an immune response. PD-L1 expression and tumor mutation burden (the amount of mutations that are present in a tumor's DNA) are the most commonly used biomarkers that predict NSCLC response to immunotherapy. For instance, anti PD-1/PD-L1 inhibitors tend to be more beneficial for individuals with high PD-L1 levels; likewise, higher tumor mutation burden (TMB) is correlated to a higher presence of neoantigens, tumor-specific antigens produced by malignant cells, which further allows for ICIs to work more effectively. However, low PD-L1 expression or low TMB can reduce response to immunotherapy by making it much more challenging for the immune system to distinguish tumors. Various genetic mutations can allow tumors to escape the immune response; however, they can also function as biomarkers which help T cells and B cells effectively identify tumors. Mutations in essential genes, such as RET and HER2, can assist

tumors in resisting treatment, despite expressing PD-L1. Some genes, including ROS, MET, and ALK, can simultaneously downregulate TMB while upregulating PD-L1 expression (Zhou et al., 2023).

EGFR, a common NSCLC mutation, was also found to elevate PD-L1 expression through signaling pathways in the TME (Zhang et al., 2016). PD-L1 upregulation is often found to weaken the immune response of T cells. Inversely, some mutations, such as KRAS, can cause different reactions in the immune system depending on an individual's PD-L1 and TMB levels. For example, STK11 is a KRAS mutation which was found to have a strong correlation to primary resistance of PD-1 and PD-L1 ICIs in NSCLC (Judd et al., 2021). Neoantigens are unique antigens that are formed through DNA mutations that happen in malignant cells. These antigens often serve as biomarkers which help immune cells identify tumorous cells and mark them as targets. However, through acquired resistance, neoantigen expression is often lost over time; without neoantigens, it can be much more difficult for immune cells to recognize the difference between healthy and cancerous cells.

APCs play an essential role in the immune system; they regulate antigen presentation that is needed for T cells to accurately identify whether or not a cell is cancerous. As previously mentioned, some tumor mutations lead to neoantigens, which help immune cells identify malignant cells. These neoantigens must be presented to T cells through human leukocyte antigens (HLAs), a specific form of MHC proteins. Loss of HLA can lead to poor antigen presentation, and as a result, immunosuppression. Recent research has shown that there's a strong association between some types of inherited HLA and the responsiveness of metastatic NSCLC cells to ICIs. Additionally, it was found that 40% of NSCLC has allele-specific HLA loss over time; this can cause upregulation of PD-L1, which leads to further resistance of ICIs (McGranahan et al., 2017). Besides HLA, there are also many other genes and proteins that are needed for proper antigen presentation. For instance, β2M , a biomarker protein, assists HLA in antigen presentation, while IFN-y, a cytokine known as interferon gamma, helps boost HLA levels, making tumors more identifiable and enhancing immune response. Loss of either of these can create further challenges during antigen recognition.

As indicated earlier, EGFR and KRAS are two common mutations in NSCLC that upregulate PD-L1 expression and inhibit immune response; both of these mutations tend to be found in signaling pathways. Signaling pathways are in charge of the regulation of cell functions, such as growth, apoptosis, and cell cycle progression. Pathways including mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (P13K) are crucial for immune cells to properly function. However, tumors tend to develop multiple mutations in these pathways, allowing them to avoid apoptosis and undergo metastasis and unregulated proliferation (Zhou et al., 2023). Targeting abnormal signaling pathways can prevent tumors from resisting treatment through specific mutations. Overall, genetic mutations that have an effect on neoantigen, PD-L1, and TMB expression in an individual can play a major role in a tumor's responsiveness to immunotherapy treatment, especially for a complex disease like NSCLC.

Methods to Combat ICI Resistance

Novel Immune Checkpoint Molecules

An investigational trial studied patients with advanced squamous-cell lung carcinoma and compared nivolumab and docetaxel to determine which treatment was safer and more effective. The median rate of survival was 3.2 months longer for the nivolumab therapy and the HR was 0.59, indicating a 41% reduction in risk of death compared to the docetaxel. To add on, it was found that the PD-L1 didn't have a predictive effect on the ICI. This indicates that PD-L1 levels in an individual had no correlation to the effectiveness of the nivolumab treatment (Brahmer et al., 2015). In immunotherapy, researchers often associate PD-L1 levels with how well patients may respond to treatments. Elevated PD-L1 expression may indicate better responses to PD-1 and PD-L1 inhibitors, depending on other factors in the individual. However, in this case, the PD-L1 did not predict how well each individual responded to the treatment. This lack of correlation suggests that the nivolumab may be effective across a broader range of patients, including those showing low PD-L1 expression. It also indicates that PD-L1 is not the only factor for determining the

effectiveness of ICIs, and opens up a possibility for other immune checkpoint molecules to be targeted through ICI therapy as well.

Currently, all of the FDA-approved ICI treatments only target the molecules PD-1, PD-L1, or CTLA-4. However, apart from these, there are other inhibitory immune checkpoints which also work to regulate T cell response. Blocking these additional molecules, either as a monotherapy or as a combination therapy with PD-1/PD-L1 ICIs, can further prevent tumors from resisting treatment. There are several new checkpoint molecules that are currently being studied as potential targets for ICIs, including VISTA, LAG-3, TIGIT, TIM-3, NKG2A, Galectin-3, B7-H6, HHLA2, and more (Figure 2). So far, VISTA, LAG-3, TIGIT, and TIM-3, have shown favorable results in numerous clinical and preclinical trials.

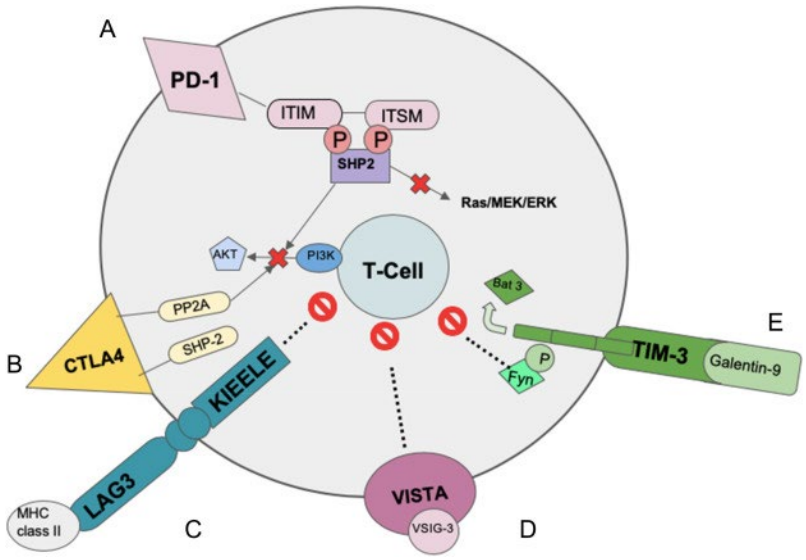

Figure 2. Diagram of immune checkpoint molecules and their signaling pathways to T cells. Source: Barrueto et al., 2020

TIGIT, or T cell immunoreceptor with immunoglobulin and ITIM domain, is an inhibitory receptor expressed on natural killer cells (NKs), T cells, B cells, and dendritic cells, which helps them recognize and attack cancer cells. TIGIT shares approximately 20% of the CD226 protein's amino acid sequence, allowing it to easily bind to its ligands, CD155 and CD112. This can lead to TIGIT overexpression, a prominent observation in NSCLC and many other cancers, inhibiting T cells from properly functioning. Inhibiting TIGIT can prevent inhibition of CD8+ cell responses in order to enhance the anti-tumor immune response (Harjunpää et al., 2019). Research indicates that combining blockades of TIGIT with existing therapies, such as anti-PD-L1/PD-1 ICIs, can be much more effective than inhibiting it as only a monotherapy. Currently, many researchers are working to produce anti-TIGIT mAbs. A recent clinical trial studied the safety of efficacy of tiragolumab, a TIGIT ICI agent, combined with atezolizumab (anti PD-L1 ICI) as treatment for metastatic NSCLC patients (Rodriguez-Abreu et al., 2020). The results proved that the combination was tolerated fairly well by patients and had a HR of 0.57, which indicated a 43% decrease in death risk compared to the atezolizumab as a monotherapy.

LAG-3, or lymphocyte-activation gene 3, is a checkpoint receptor protein that is found on B cells, T cells, dendritic cells, and NK cells. When LAG-3 levels are increased, it can cause the immune response to weaken. By inhibiting LAG-3, T cell exhaustion can be prevented, allowing cytokine production to be boosted as well. LAG-3 shares almost 20% of the CD4 protein's amino acid sequence, allowing it to bind strongly to MHC class II molecules and block TCR signaling, which is necessary for activation of CD4 cells by an APC. Additionally, its interactions with other ligands in the tumor microenvironment (TME), the complex tumor ecosystem within the body, further

contributes to T cell exhaustion (Chocarro et al., 2021). Researchers believe that targeting LAG-3 during treatments is crucial in order to enhance immune responses within individuals. So far, two LAG-3 agents, relatlimab and eftila gmod alpha, have been FDA-approved to be used in combination with PD-1 inhibitors and many more are still being explored (Ibrahim et al., 2023).

TIM-3, or T cell immunoglobulin and mucin-domain 3, is another emerging immune checkpoint which is expressed on T cells, regulatory T cells (Tregs), dendritic cells, NK cells, and B cells, and it also works to maintain proper immune responses. TIM-3 shares about 25% of the CD4 protein's amino acid sequence, which allows it to effectively interact with its ligand Galectin-3. In some cases, this can cause TIM-3 upregulation, which can promote T cell exhaustion in the TME and limit immune cell ability to produce cytokines that are necessary for an effective immune response (Zhu et al., 2010). A recent study involving two adenocarcinoma patients expressing PD-1 resistance showed that both patients were expressing elevated levels of TIM-3. Additionally, the scientists also studied the TME of mice and found that the cells which were previously treated with ICIs were found to have higher TIM-3 expression than the cells which weren't treated (Koyama et al., 2016). The data indicated that high expression of checkpoints, such as TIM-3, may have a strong correlation to acquired resistance to PD-1 inhibitors in adenocarcinoma. Inhibition of these molecules may potentially reduce PD-1 resistance in NSCLC.

VISTA, or V-domain immunoglobulin suppressor of T cell activation, is a checkpoint that is usually found on T cells, Tregs, and dendritic cells. Upregulation of VISTA can cause the immune system to become less responsive to foreign antigens; this is known as immune tolerance. In addition, VISTA and PD-L1 share many functions and have approximately 22% of the same amino acid sequence, indicating that VISTA may also be a potential target for ICIs (Tagliamento et al., 2021). A recent study found that high levels of VISTA have high association to low levels of TMB and loss of EGFR mutations in adenocarcinoma. VISTA was also found to be expressed in 99% of NSCLC cells that had a predominant cytoplasmic or membranous staining pattern. (Villarroel-Espindola et al., 2018). Because it is so commonly expressed in NSCLC and shares many similarities with PD-L1, researchers believe that targeting VISTA may have multiple benefits for a wide variety of patients.

Combination Therapies

Combination therapies refer to treatment strategies which incorporate the use of two or more forms of treatment in order to reduce the likelihood of treatment resistance by targeting. the cancer from multiple aspects. Lung cancer is considered to be a complex disease due to several interrelated factors which contribute to the disease's progression and development. Often, genetic mutations and alterations can influence the interactions that occur in the TME, resulting in the activation and infiltration of effector immune cells. Additionally, lung cancer can be extremely heterogeneous, with different cells expressing different reactions to treatments. Combination therapies involving ICIs and other modalities such as chemotherapy, radiation therapy, or targeted therapies, can work to better combat resistance in NSCLC. In 2021, a phase II trial evaluated the effectiveness of combining durvalumab, an anti-PD-L1 ICI, with stereotactic body radiotherapy for individuals with early-stage NSCLC. The trial randomly assigned 60 patients to receive one of the two treatment methods for over three weeks. Results indicated that the major pathological response, the percentage of the tumor which is no longer viable, was significantly higher in the combination patients (53.3%) compared to the monotherapy patients (6.7%). 50% of the patients in the combination therapy group achieved a complete response from the treatment and patients in both groups experienced AEs (Altorki et al., 2021). Another study compared the efficacy of ICIs alone and combined with chemotherapy for individuals with advanced NSCLC and high PD-L1 expression. The results showed that the combination therapy had a 28.3% increase in objective response rate (ORR) and a 28.4% increase in the disease control rate (DCR), as well as an increase in PFS and decrease in risk of death. It was also observed that the patients in both the combination and the monotherapy had similar scores of tumor burden, indicating that the addition of the chemotherapy did not have any major negative effects on the treatment's tolerability (Wang et al., 2021).

Results

The goal of this literature review was to explore potential methods involving immune checkpoint inhibitors to overcome treatment resistance in NSCLC tumors. Research found that common resistance mechanisms for NSCLC cells include genetic mutations, interactions between cells in the TME, and specific molecule levels that are unique to individuals. As of now, the primary methods being studied to improve treatment includes combination therapies, involving immunotherapy combined with one or more forms of treatment, as well as the usage of ICIs, to target newer checkpoint molecules. Numerous clinical trials have shown promising results for these newer treatments, especially because they build on existing immunotherapies in order to further strengthen their effectiveness. Further research and development of these strategies can truly have a major impact on NSCLC patients worldwide.

Conclusion

In conclusion, overcoming barriers in ICI treatment for lung cancer can substantially increase survival rate in patients around the world and also open up future exploration areas for breakthroughs in cancer research. Being one of the deadliest cancers, lung cancer is a crucial issue that researchers around the globe are working to mitigate. Immunotherapy, particularly with ICIs, appears to be a promising treatment that can significantly change the outlook for patients suffering with various types of cancer.

ICIs work by removing checkpoints which inhibit T cell function and can prevent T cells from attacking cancerous cells. Rather than directly destroying malignant cells, ICIs have the ability to enhance the immune response and allow the immune system to target tumors using its natural processes. Malignant cells have the ability to evolve and resist treatments and therapies through many mechanisms. Many methods are being studied to overcome ICI resistance in NSCLC patients. By developing new checkpoint molecules for ICIs to target, researchers can prevent tumor resistance to inhibitors that attack PD-L1/PD-1 or CTLA-4 checkpoints. Checkpoint targets such as VISTA, TIGIT, LAG-3, TIM-3, and more are currently being investigated as possibilities for the future. Furthermore, by utilizing combination therapies that involve ICIs and another form of treatment, tumors can be more effectively recognized and attacked from multiple points, simultaneously. Combining ICIs with radiation, chemo, targeted therapies, or even other forms of immunotherapy, will allow multiple actions against the tumor to take place at the same time, making it more difficult for the tumor to resist the treatment.

Limitations

Though there are many new strategies being presented to combat ICI resistance in NSCLC, there are still many factors that have to be considered before these can actually be implemented on real patients. Particularly with combination therapies or the development of novel checkpoint targets, it is important to note that many of these therapies can lead to higher rates of severe AEs and immune-related toxicities. Since each individual has different genetics, mutations, and immune responses, it can be difficult to understand how different patients will react to newer treatments. Though these concerns may be minor, they are still worth mentioning, as they may lead to serious considerations for future patients and treatment outcomes. Furthermore, this review was developed entirely through the use of online research. Despite having access to a substantial amount of data from previous trials and articles online, the research lacked physical evidence and data. This created challenges, especially when trying to retrieve up-to-date information on a specific concept.

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