

Applications of immune checkpoint inhibitors (ICIs) in the medical fields

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Abstract. ICIs are a kind of immunotherapy that works by preventing immune checkpoints from functioning normally, which are essential immune system components. ICIs are currently the most used immunotherapy regimen. Based on the patient's health, the cancer type, the length of the illness, and the dose of inhibitors the patient can tolerate, the therapy can cause side effects of indeterminate duration and varying degrees. However, the therapy remains beneficial for patients. Therefore, the effects of immunotherapy on the human body are still an issue that needs to be explored. An overview of ICIs in immunotherapy will be given in this paper, including the following concepts: (i) General information on treatments with immune checkpoint inhibitors (ii) The immunotherapy's mechanism and application (iii) Problems and complications with ICI therapies (iv) Ways that the immunotherapy can be improved and the future direction of ICI.

Keywords: Immune Checkpoint Inhibitors (ICIs), Mechanism, Anti-PD Antibodies, Anti-CTLA-4 Antibodies.

1. Introduction

Cancer treatment has been a heated topic in the healthcare system for several decades. About one in eight people die yearly because of cancer [1]. Fortunately, scientists discovered a lot of new information on mutations in cancer genes, including why cancers happen. In most cases, alterations in the cancer cell genome, specifically in DNA sequences, will lead to the occurrence of cancer [1]. As a disease caused by genomic abnormalities, when cancer occurs, the patient's genome becomes unstable and is accompanied by a large number of point mutations [2]. One of the most obvious characteristics of cancers is that they can proliferate themselves infinitely and have the ability to invade normal human organs [1]. The immune system, as an automatic defense system in the body, functions in the process of identification and eradication of cancer. This is mainly due to innate cells in the immune system such as NK cells, eosinophils, and basophils as well as phagocytes are present in tumor microenvironments which slows down the tumor progression [2]. Therefore, immunotherapy, a new paradigm of cancer treatment, has been invented and has achieved great success since its discovery. Although the idea of eradicating cancer by increasing the activity of the host immune system was proposed a century ago, it has not been realized until recent decades [2]. This has certainly changed the inherent approach to clinical cancer treatment and has made a huge difference to the entire field. Immunotherapy can be divided into three main types through the principle of treatment, they are passive immunotherapy, active immunotherapy, and immunomodulatory therapy. The principle of passive immunotherapy is to kill the tumor cells in the patient's body by passively receiving antibodies or T cells injected from outside; active immunotherapy is to kill the tumor cells by activating endogenous cells similar to cytokines; and immunomodulatory therapy is to kill the tumor by changing the microenvironment around the tumor, such as the inhibitors will be mentioned in this article. Compared to traditional treatments targeting tumor cells directly, immunotherapy focuses more on reactivating the patient's suppressed immune system, which is considered a more effective therapy for individuals suffering from metastatic cancer.

Immunotherapy's success in treating cancer means that the status of immune cells can be artificially altered. Taking T-cells as an example, on account of random recombination of the T-cells receptors genes, the body produces a diversity of distinct T cells, for instance, regulatory, helper, and cytotoxic T cells, which all contribute to the body's immunological response, and studies have shown

that their activity can be altered through checkpoint inhibitors. However, the immune checkpoint inhibitors were not the first immunotherapies to be invented. Long before that, in 1892, William Coley discovered that hybrid cells from *Serratia marcescens* and *Streptococcus pyogenes* may result in tumor mortality, which was the prototype of the oncolytic virus therapy [2]. Since then, new immunotherapies are always being developed, including cancer vaccines that use tumor-specific antigens to elicit T-cell immune responses, cytokine therapies that effectively disseminate immune signals through cytokines, and adoptive cell transfer therapies that eliminate cancer cells by enabling ex vivo immune cell expansion and patient reinfusion (Figure 1) [2]. Until 1955, ICIs were first discovered by American immunologist Jim Allison. T cells are essential for the innate immune system's efficient eradication of cancer cells. However, T cells' reactivities may harm healthy tissues without proper control. By sending signals to T cells, checkpoints function as immune system regulators to modulate immune responses. Notice that immune checkpoint inhibitors are only one option out of many available immunotherapies, different immunotherapies should be appropriately applied to various forms of cancer.

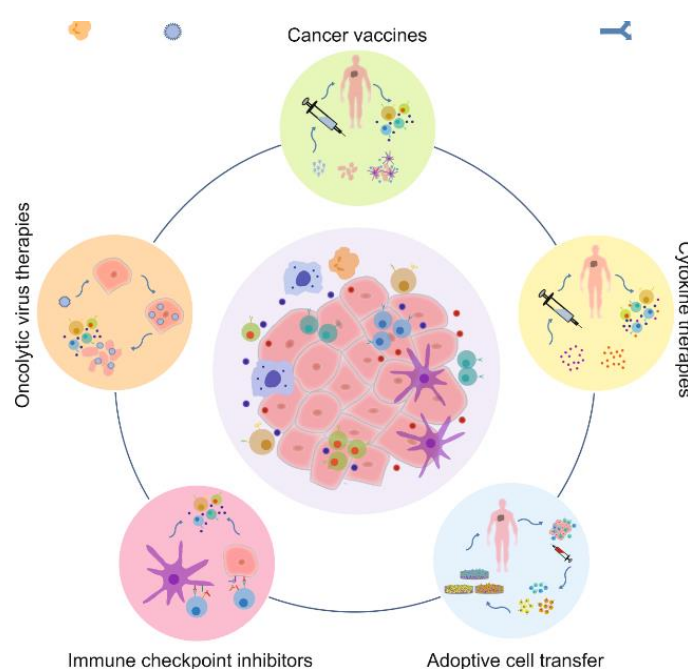


Figure 1. immunotherapies have been discovered [2].

Nowadays, increasing the function of a specific immune system component in cancer victims through checkpoint inhibitors has become one of the viable treatment modalities, and continues to provide valuable resources for clinical cancer research. Certainly, the checkpoint inhibitor is significant to the realm of therapies for cancer. ICIs' application is becoming more and more widespread, but its emergence has undoubtedly brought more issues for scientists to explore. This review aims to provide a systematic review of articles about ICI applications and summarize some of the current key applications.

2. General Information on Immune Checkpoint and Immune Checkpoint Inhibitor

The presence of immune checkpoints, a critical component of the immune system as a whole, prevents the system from attacking the body's cells indiscriminately and causing autoimmune conditions. There are two kinds of immune checkpoints, the first the stimulatory checkpoint molecules, and the other the inhibitory checkpoint molecules. In this case, immune checkpoint inhibitors are used to target inhibitory checkpoint molecules in cancer treatment. Take T-cells as an example, when the proteins on its surface recognize the proteins on other healthy cells and interact with them, the immune checkpoints will start to work. They send a biological signal to the bound T

cell, causing it to stop exerting effector functions. These receptors usually use single tyrosine signaling motifs to deliver inhibitory signals, such as the motif of immunoreceptor tyrosine-based switch and the immunoreceptor tyrosine-based inhibitory [3]. Some tumor cells take advantage by stimulating these checkpoints, forcing them to send signals to lymphocytes. The immunotherapy drug, immune checkpoint inhibitors, could prevent the T-cells' checkpoint protein from adhesion to the target cell protein and thus destroy cancer cells.

Checkpoint inhibitors are currently the most successful antibody-based therapies. Although the history of antibodies goes back a long way, it was not until the 1970s that a large number of more in-depth studies emerged, and monoclonal antibodies were one of them. As the "predecessor" of ICI, monoclonal antibodies are man-made molecules designed to be used as substitutes for endogenous antibodies that can recover or mimic the operation of immune cells against unwanted cells, such as cancer cells. The earliest monoclonal antibodies were created by German biologist Georges Köhler and Argentine biochemist César Milstein, two scientists who got the idea from Cotton's myeloma cell fusions. Shortly after that, the ICIs were discovered.

Over the past few decades, through continuous research on ICIs, scientists have identified various inhibitory receptors in the immune system like LAG3, PD-1, CTLA-4, BTLA, and TIM3. Checkpoint PD-1 helps to stop T-cell from damaging normal cells when binding to its ligand called PD-L1[3]. Similarly, when CTLA-4 binds to B7, the ligation prevents T cells from carrying out effector functions. In other words, when cancer cells are recognized, T cells won't be able to take action. More notably, BTLA, known as B and T Lymphocyte Attenuator, another checkpoint, is present on a variety of immune cells, examples would be dendritic cells, T and B cells, NK cells, and macrophages. It also plays a suppressive role in various diseases (e.g., high levels of BTLA are expressed in patients who have cancer). Till December 2021, there are already 8 approved drugs that aim for 17 different malignant tumors. Immune checkpoint inhibitors' development has undoubtedly aided in the fight against cancer. However, despite hundreds of ongoing and successful clinical trials worldwide, the results showed that ICIs only help a tiny proportion of patients [4]. The main reason for this is that immunological responses to the immune system will be produced from the tumor microenvironment, and different phenotypes of tumors have different mechanisms to prevent the eradication process by immune cells [4]. Besides that, this immunotherapy also brings side effects to patients treated, some of the symptoms that may occur are diarrhea, fatigue, cough, nausea, rash, anorexia, constipated ion, and muscle and joint pain. More serious ones may be infusion reactions (allergic reactions after taking the drug) and autoimmune reactions (T cells out of control and attacking healthy cells indiscriminately). Whether which checkpoint inhibitor it is, all of them share a common characteristic: they are extremely different from other cancer therapies and are likely to have some irreversible effects on patients. Furthermore, it also appears important to identify the patients who can be treated with this kind of immunotherapy, for example, the use of anti-PD drugs or CTLA-4 inhibitors on patients. As mentioned before, to make the immunological response more intense, immunotherapy modifies the status of T lymphocytes, allowing patients suffered fewer side effects. However, the effects of these immunotherapies often take longer to the observation of treatment effects compared to targeted therapies, which may lead to the missing optimal time of patients for therapies [5]. Fortunately, analyses of peripheral blood may be used to anticipate how will the therapeutic effect of immunotherapy going to be [4]. Primarily by comparing the diversity and range of T cell receptors in patients' peripheral blood to get predictive biomarkers, thus finding the most appropriate patients for immunotherapy [5]. ICIs have been used to treat lung cancer, cervical cancer, breast cancer, etc.

3. Immune Checkpoint Inhibitor Mechanism and Applications:

3.1. T-cell Activation Process

In order to trigger the body's immunological response, naïve T cells must be activated. As the main mediator of the activation process, T cell receptors (TCR) ensure the specificity of each cell [6]. More specifically, TCRs will recognize and interact with antigen-presenting cells (APCs) with antigenic

peptides (after interacting with either class of the major histocompatibility complex) as well as the co-receptor CD4/CD8, these three components work together to form signal 1 in the activation process (Figure 2) [6,7]. However, only stimulation of signal 1 may lead to activation failure for T cells. Signal 2 as a co-stimulation signal can avoid such problems, this is supported by a study, scientists confirmed that T cells require dual signaling for activation by inactivating CD28 (cause loss of function to interact with APC) [6]. CD28 interacts with B7 to deliver this signal to T cells [7]. In short, these two signals working together can make sure to keep T cells away from a non-responsive state. In addition, IL-2 is mainly responsible for T cell differentiation and homeostasis, but it does not respond to naive T cells (Figure 3), so signal 1 and signal 2 are responsible for activating IL-2 to make T cells grow and thus activate T cells [8].

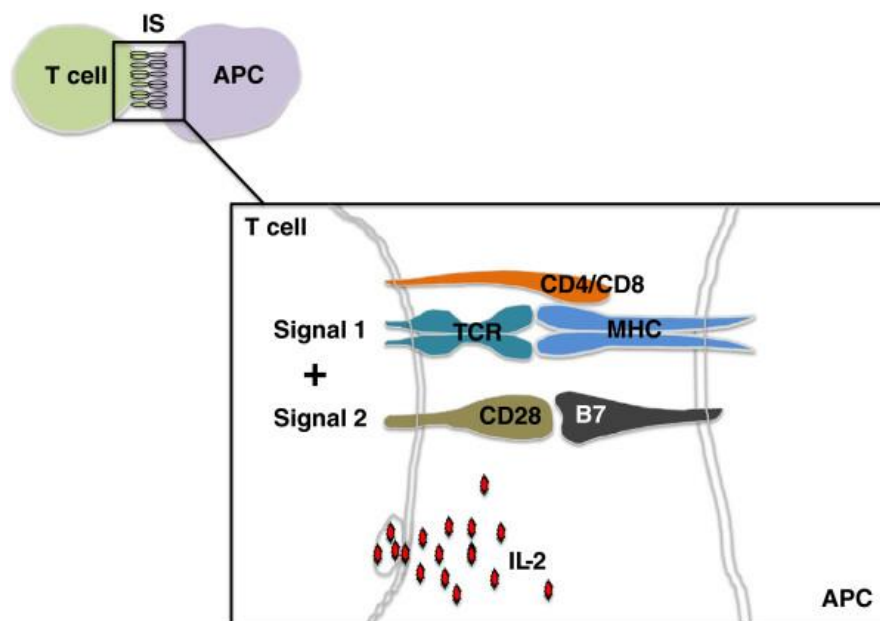


Figure 2. During T cell activation, signals 1 and 2 [7]

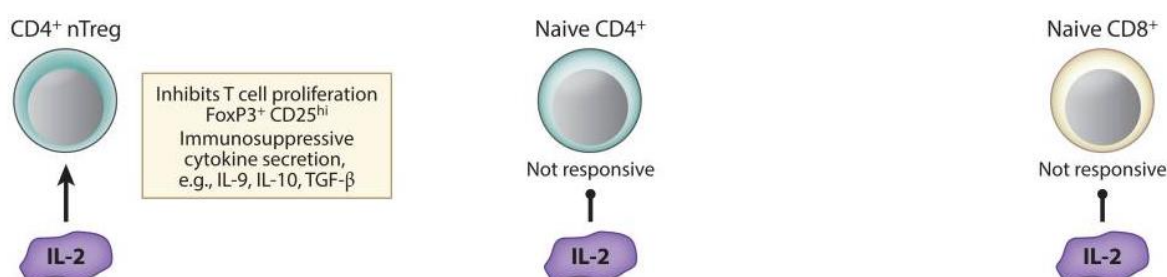


Figure 3. IL -2 activity on the Naive T-cell [8].

3.2. Anti-CTLA-4 antibodies and CTLA-4

One of the immunological checkpoints involved in controlling immune responses is CTLA-4, also known as CD152, the complete name of which is cytotoxic T lymphocyte-associated antigen. From the structural aspect, CTLA-4 is similar to stimulatory protein CD28 which is involved in the execution of signal 2. CTLA-4, however, has a significantly stronger affinity to CD86 and CD80 (APC B7 ligand) and this is why CTLA-4 can be a suppressive checkpoint, more specific, both CTLA4 and CD28 can bind CD80 rapidly, and an experiment showed that their dissociation rate constant (k_{off}) is fast which reached $K_{off} \geq 0.43$ and $K_{off} \geq 1.6$ respectively, this allows these two receptors to compete for combination at any time [9]. Once the binding between CTLA-4 and CD80/CD86 has complete. it prevents CD28 from binding to B7 ligands to send proliferative signals to T cells during the activation phase [10] (Figure 4), thus achieving the goal of inhibiting T cells when

they are overactivated. Besides that, another characteristic of CTLA-4 is that it will be endocytosis which 90% of the receptor is inside the T cell [11]. The output of large numbers of endogenous T cells is necessary to fight human disease, but inevitably some T cells will generate a self-reaction and when these cells enter the peripheral pool CTLA-4 takes care of the regulatory work [11]. Furthermore, the cytoplasmic tail of CTLA-4, which is 36 amino acids long, contains two tyrosine-based residues [11,12]. These two residues are located in the cytoplasmic tail in the motifs YVKM and YFIP in the Y201 and Y218 positions, respectively. It was demonstrated that although the YVKM motif does not play a substantial role in CTLA-4 function, the motif is still beneficial for CTLA-4 blockade signaling. A study in 2000[12] also confirmed this claim by introducing CTLA-4 mutants to mice who lack CTLA-4, including the mutant CTLA-4 Tyr 201 residue of CTLA-4 (Y201V), another mutant CTLA-4 lack of the YVKM residue (Δ CTLA-4 tail), and the wild-type of it (FL) and observing their in vivo T-cell expression[12]. Mice injected with the Y201V mutant were found to boost the surface expression of CTLA-4 on T cells, which later confirmed that consistent with CTLA-4 endocytosis, in contrast, after receiving an injection of Δ CTLA-4 tail, mice's surface CTLA-4 expression did not increase which certainly indicates the importance of the residue (YVKM) for the proper functioning of CTLA-4[12].

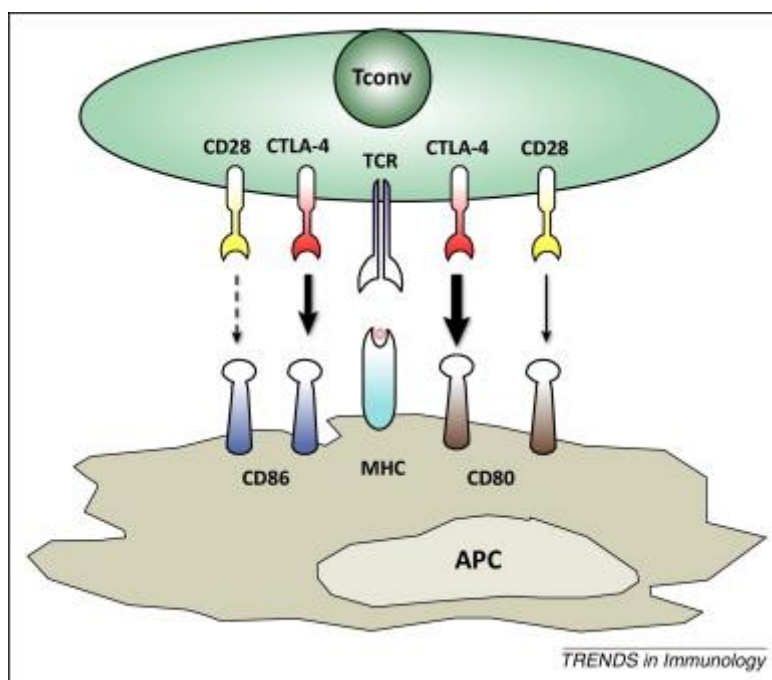


Figure 4. CD28 and CTLA-4 binding to CD86 and CD80 [11].

Normally CTLA-4 suppresses the immune response by blocking the B7-CD28 interaction, and the presence of this signal reduces T-cell appreciation [13]. Similarly, in tumor-draining lymph nodes, CTLA-4 also acts as a blocker of APC and T cell interactions [13]. So anti-CTLA-4 (e.g., ipilimumab) is used to block the continued action of CTLA-4, allowing B7-CD28 interaction to release T cell value-added signals and thus induce an immune response. Inside the tumor environment, it was discovered that Treg cells have higher levels of CTLA-4 expression compared to intra-tumoral Teff cells, which may also lead to a preferential action of anti-CTLA-4 on Treg rather than Teff[13]. Regardless, James P. Allison et al. reported back in 2009 that both Treg and Teff would be the therapeutic targets for anti-CTLA-4 drugs.

The most prevalent kind of cancer, liver cancer, claims about 700,000 lives annually throughout the world, the widespread usage of ICI has resulted in a significant change in how the illness is handled. Sorafenib, the only approved anti-liver cancer drug worldwide, has not performed well in monotherapy, so the addition of ICI therapy has improved the situation for liver cancer patients now. Tremelimumab, one of the CTLA-4 inhibitors, was the first ICI used to treat advanced hepatocellular carcinoma (HCC) [14]. It is a monoclonal antibody and helps prevent the CTLA-4-B7-induced

downregulation of T-cell activities.[15]. In a study [14] on how Tremelimumab will affect liver cancer, 17.6% of patients showed partial remission after getting treated, 58.8 percent showed stabilization of the disease, and the disease control rate reached 76.4 percent [14]. This certainly shows that ICI can benefit patients with liver cancer. Another study [15] also demonstrated the impact of Tremelimumab in combination with other therapies in advanced HCC patients, 32 people in all were enrolled in this trial, and they will receive Tremelimumab regularly and one ablation treatment during the trial [15]. Of the patients who could be evaluated, 26.3 percent showed remission, with survival rates of 57.1 percent and 33.1 percent at 6 and 12 months after the trial, respectively, if side effects from the therapy were ignored [15]. Undoubtedly, these data reveal the potential of drugs against CTLA-4 in liver cancer treatment.

3.3. Anti-PD antibodies and PD-1/PD-L1

PD-1 and PD-L1 have almost the same function as CTLA-4 in the system which is also in charge of peripheral tolerance maintenance. Although PD-1, as the suppressor checkpoint in the immune system, shares a 20% amino acid sequence similarity with CTLA-4, it differs from CTLA-4 in that it is not just found on T lymphocytes but also NK cells, monocytes, and so on [16]. PD-L1 as the ligand of PD-1 belongs to the B7 receptor family as well and may be seen in B cells, epithelial cells, T cells, and macrophages [16]. However, PD-L1 is also produced in cancer cells and achieves tumor immune escape by binding to T cell receptors or by creating T cell lysis resistance in tumor cells, among other mechanisms [17]. The activation, value-added, and other T cell activities are suppressed by the interaction between PD-1 and its ligands, not only on a daily basis but also in tumor microenvironments, which lessens the autoimmune reaction. (Figure 5) [16].

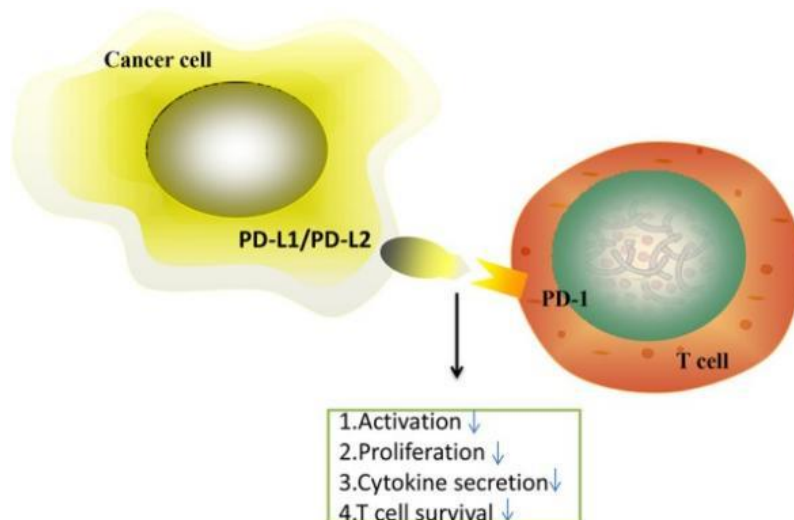


Figure 5. The binding procedure of the checkpoint [16].

Both Anti-PD-1 and PD-L1 drugs are used in checkpoint activation blocking. Although PD-1 belongs to the same family as CTLA-4, however, anti-PD therapy's mechanism is completely different from the CTLA-4 inhibitor [18]. CTLA-4 blockers are more oriented to modulating the overall T-cell response, while anti-PD therapy focuses more on selective modulation of the response within the tumor microenvironment [18]. PD-1 expression level will be elevated when the tumor-influencing interferon-gamma (IFN- γ) is presented and allows T lymphocytes to receive more inhibitory signals for immune escape [19]. When the situation is happening, PD-1 inhibitors are used to block PD-1, primarily by overlapping and interacting with the PD-1 surface thus acting as a suppressing force [19]. So far, except for the representative Nivolumab and pembrolizumab, lots of other Anti-PD-1 medications are still during the phase of clinical trials [19]. However, the effect of Anti PD drugs in monotherapy is not so significant, and one study showed that the usage of surface anti-PD drugs may induce IL-10 levels to rise [20]. IL-10, as a cytokine, also inhibits T lymphocytes,

which could contribute to the continuation of cancer immunosuppression, so the PD-1 inhibitors and IL-10 neutralizing drugs work together can effectively slow down the progression of cancer [20].

Not only can Anti-CTLA-4 benefit patients with liver cancer, but Nivolumab, an inhibitor for PD-1, may also use in treating hepatocellular carcinoma. A 2017 research [21] demonstrated that 77% of all 262 patients enrolled in the clinical trial who got separated into the phases of dose expansion and dose escalation completed the entire treatment, and the drug demonstrated acceptable safety and tolerability during the dose escalation phase (3.0 mg/kg was the highest dosage given to part individuals. Others received a maximum dose of 10 mg/kg) [21]. Patients receiving 3 mg/kg Nivolumab during the dosage-expansion phase had a 20% remission rate and 15% during the phase of dose escalation, however, some patients demonstrate the symptoms of an increase in alanine aminotransferase, rash, and a higher level of aspartate aminotransferase, etc. after getting treated [21]. The relationship between these treatment-related adverse effects and dose size was uncertain [21]. Furthermore, other approved drugs are also effective for lung cancer treatments. A study in 2020[22] showed that the combo of the PD-1 inhibitor Sintilimab with placebo was effective in rising the survival chance of non-small-cell lung cancer patients during the progression-free stage, with a remission rate of 51.9% with this combination therapy [22]. Additionally, the application of Nivolumab to individuals with resectable early-stage NSCLC may stabilize the disease with fewer side effects and without delaying surgery, nine out of 20 patients (45%) had a major pathological response after getting treated [23].

3.4. Anti-LAG-3 Antibody and LAG-3

LAG-3, a new inhibitory receptor, is involved in lymphocyte activation. It is one of the members of the immunoglobulin superfamily that resembles CD4 in structure.[24]. However, it has a greater affinity to MHC-II on the APC than CD4, preventing the MHC molecule from interacting with the TCR and also CD4[25]. LAG-3 is mostly present in Treg Cells, not only that, but it is also produced in intraepithelial lymphocytes, and natural killer cells, however, on naïve T cells, the checkpoint won't express [24,26]. The inhibitory qualities of LAG-3 correlate with its amount of expression on immune cells' surfaces, and its inhibitory effects vary for different lymphocytes, so as a co-inhibitory receptor, it is distinct from CTLA-4 and PD-1 [25,26]. This receptor certainly maintains the immune system's stability by preventing T cell activation and secretion of cytokines [25]. Although CD4 and LAG-3 are akin, LAG-3 inhibits T cells by a novel mechanism that inhibits signaling through intracellular region transduction, and has been proved that it is not involved in competition with CD4 for pMHCII binding [26]. Structurally speaking, LAG-3 also has a distinctive cytoplasmic tail. It comprises three distinct conserved domains, including a KIEELE motif, a repeat of glutamic acid-proline, and a serine phosphorylation site [25]. The KIEELE sequence is responsible for signaling to prevent T cells from entering the S stage of the cell cycle and thus achieving inhibition, while the EP is responsible for the localization of LAG-3[25].

Up to September 2020, 13 drugs targeting LAG-3 have been developed and are divided into three main categories according to their therapeutic, Anti-LAG-3 bispecific medications, monoclonal antibodies, and LAG-3-immunoglobulin fusion proteins [26]. Among them, soluble fusion proteins arouse the immune response by activating antigen-presenting cells (APCs), while monoclonal antibodies still enhance the immune system activity by traditionally preventing LAG-3 from attaching to its ligand. The application of LAG-3 is mostly in immunotherapy as an adjunct to other ICIs in combination therapies, such as the combo of an Anti-PD medication spartalizumab with the Anti-LAG-3 drug ieramilimab (LAG525) for more effective stabilization of patients with malignancies and the combination of eftilagimod alpha(efti) with another drug called pembrolizumab to treat individuals with advanced melanoma [27].

4. Problems and complications with ICI therapies

4.1. Resistance to ICIs

The use of ICI in clinical cancer treatment has resulted in longer survival times for cancer patients. However, long-term follow-up records of patients participating in clinical trials indicate that late-stage cancer recurrence continues to occur, and the presence of this phenomenon undoubtedly indicates the instability of tumor cells and the development of drug resistance. Patients treated with ICI can be broadly categorized into three types: people who first respond to the medicine and keep responding to it; patients who don't react to the drug at all (innate resistance); and individuals who successfully respond to the therapy at the start but eventually, their sickness gets worse (acquired resistance). Taking PD-1 as an example, 160 trial findings from a meta-analysis revealed that although PD-1 inhibitors improved the survival rate of both patients with PD-L1 positivity and negativity, the initial efficacy was about 10%-20%, this is an example of innate resistance [28].

4.2. Immune-related adverse events(irAEs)

The one side effect that is exclusive to ICI immunotherapy is immune-related adverse events (irAEs) which happen when the immune system becomes overactive. irAEs can occur in any organ in the body, and organs with a higher likelihood of irAEs are the skin, muscles, intestines, lungs, endocrine system, and skeletal system. As a result of ICI, suppressed immune cells become active again and indiscriminately damage benign cells and malignant cells, leading to serious side effects. Combination therapy with ICI and other treatments can be effective in reducing patient resistance, but with it comes a higher rate of toxicity and incidence of irAEs. For instance, the interaction of nivolumab and ipilimumab, significantly prolonged overall survival in individuals with melanoma, however, the rate of occurrence of irAEs is up to 93% (509 patients out of 547 patients) [29].

5. Future directions of ICIs

5.1. information expansion and preclinical biomarker test

Based on the existing studies, ICIs perform well in cancer treatment, but the dose of the drug and the target tumor microenvironment is still not precise enough, so in the future, ICIs can be improved in two directions. First, researchers should expand all the information and details about ICIs. Providing more detailed learning, such as information on the molecular mechanisms of T-cell-tumor cell interactions, can help researchers identify which checkpoints can be inhibited by monoclonal antibodies. From this process, researchers can also get information like what new biomarkers can be used to predict the therapeutic effect and what factors were overlooked in past research. Therefore, this is significant and this is also the basis for allowing the remaining two points to be developed realistically.

A second point where ICIs could be enhanced is to make preclinical biomarker testing mandatory for all patients about to receive ICI therapy, which could reduce the incidence of irAEs. One example is that peripheral blood analysis can be used as a biomarker [5]. Including this step in the overall treatment process allows clinicians to assess the patient's suitability for ICI therapy and the risk after completion of treatment, thus allowing each patient to receive the therapy that is more appropriate for them. Combining these two elements can make the use of ICI therapy in the medical field more personalized and safer.

5.2. ICIs and Covid-19

Almost 2 years after the outbreak of covid-19, there is yet no particular medication for the infection, and the main defense against it is immunizations. The immune mechanism seems like contributes to the increase in the death risk of cancer patients with coronavirus, but in their paper, Luo et al. [30] suggest that vaccination against covid-19 may not increase the incidence of irAEs, but rather may

enhance the efficacy of ICI therapy for cancer patients [30]. However, this statement is still waiting to be confirmed by more clinical trials.

6. Conclusion

The immune checkpoint is an indispensable factor in maintaining the immunity's homeostasis, which prevents immune cells from becoming hyperactive. Reducing suppressive activity through immune checkpoint inhibition has been a popular treatment modality and continues to provide valuable information for clinical cancer research. Unfortunately, ICI therapies can now only make patients better by making changes to the activity of the suppressor T-cell checkpoints. In addition, more refined ICI therapies require that the exact dosage of the drug be given to the patient on a specific instance basis. To achieve this, increased knowledge of the immune microenvironment, pre-treatment testing, and more precise timing of drug administration is inevitable. Up till now, the common cancers treated with ICI therapy are stomach cancer, colon cancer, head cancer, and lung cancer. This paper describes the mechanisms of specific checkpoint inhibitors based on the various immune checkpoints. It also presents the problems of Immunotherapy-related malignant events and suggests possible enhancements for the therapy in this article. There is no doubt that ICI therapy has made an undeniable contribution to the field of cancer treatment and is a topic worthy of continued research.

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