

Application of Immune Checkpoint Inhibitors in Advanced Melanoma

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Abstract. The advent of ICIs has significantly improved outcomes for many types of cancer treatment, including advanced melanoma that cannot be respected. Among melanoma patients, there is clear evidence that Ipilimumab, the first monoclonal antibody approved for CTLA-4 targeting, as well as nivolumab and pembrolizumab, which concentrate on PD-1, have led to increased overall survival. Combination therapy with other therapies shows better efficacy with ICI compared to monotherapy (e.g., a combination of CTLA-4 and PD-1 inhibitors). This article will explore recent research on the therapies of advanced melanoma, investigate the pathways of CTLA-4 and PD-1/PD-L1 and their application as single therapy or combination with other therapies such as PD-1 drugs, in addition to other therapeutic methods, and the current application of TCM treatment. In addition, this review will discuss drug resistance to ICI treatment and explore potential biomarkers associated with ICI response in advanced melanoma patients and future prospects for ICI treatment.

Keywords: Immune Checkpoint Inhibitors, advanced melanoma, CTLA-4, PD-1, irAEs.

1. Introduction

Melanoma ranks as the third most prevalent cutaneous malignancy. Although accounting for less than 5% of cases, melanoma remains a prominent and concerning type of skin cancer, contributing significantly to the mortality rate among skin cancer patients. Furthermore, it has demonstrated a rising incidence in recent years [1]. At the beginning, standard chemotherapy stands as the only therapeutic choice, with an OS rate for treated patients that below 5% over a five-year period. Previous treatment has certain limitations, some patients cannot tolerate it, poor compliance, and for some families, their financial burden is also unbearable, and there is an urgent need to find alternatives. During the last decade, cancer immunotherapy has rapidly developed, many malignant tumor clinical treatments have been customized. Particularly, the use of ICIs in treatment has brought a significant improvement in clinical outcomes of melanoma patients, showing their promising therapeutic effectiveness and treatment stability. However, due to the high heterogeneity of tumors, they can mutate to evade the immune response. At the same time, patients' predicting response and resistance to ICI is unstable, challenge the broad application of ICI. In recent years, ICI combination therapies show the ability to compensate various limitations of monotherapies. The above issues are discussed in the light of these issues.

2. Mechanism

ICIs are monoclonal antibodies that are designed to target inhibitory checkpoint proteins that are frequently overexpressed on the surface of APC and CD4⁺ T cells. By blocking checkpoint proteins from binding with their partner protein on others like tumor cells, activates T cells. ICIs enhance the immune response by regulating either inhibitory signals or negative regulation. Hence, it plays a vital role in safeguarding normal cells from being targeted by tumor cells. At present, there are 7 ICI approved by the US FDA, including 1 against CTLA-4, 3 against PD-1 and 3 against its PD-L1. There's also other ICIs are undergoing clinical trials.

2.1. CTLA-4

CTLA-4, also known as CD152, is a receptor that modulates T cell activity by interacting with B7 molecules present on surface of APCs and mediating signalling to inhibit activity. It that shares homology with CD28, a T cell co-stimulatory protein. More precisely, when both of these molecules engage with CD80 and CD86, CTLA-4 functions as a potent binding and competitive antagonist receptor, thereby inhibiting the de-stimulatory signalling of CD28 [2]. Consequently, it that downregulates the activation of T cells, promoting immunosuppression. Studies conducted both in vitro) and in vivo have provided evidence of the anticancer effects resulting from the blockade of CTLA-4 [3]. By promoting the activity and multiplication of a significant number of effector T cells, irrespective of T cell receptor specificity, it impacts the immunological priming stage of T cell activation.

2.2. PD-1/PD-L1

In current research scope, the blockade of PD-1 and PD-L1 has emerged as a pivotal foundation in the field of cancer immunotherapy. PD-1 is a surface protein for two cells at the same time, T cells and B cells. PD-1 serves to prevent autoimmune diseases by enhancing the apoptosis of antigen-specific T cells and reducing the regulatory apoptosis of T cells, a process that can, in turn, hinder the immune system from effectively eliminating tumor cells. Tumor cells often exploit in PD-1 pathway to evade the immune surveillance. They increase the expression of PD-L1, ligands for PD-1, which inhibits T-cell activity by inducing exhaustion. Reactivating a positive immunotherapy response can prevent PD-1 interact with PD-L1. Together, the primary function of PD-1 is to modulate the immune response, which can have both beneficial and detrimental effects depending on the context. In cancer immunotherapy, the aim is to counteract its inhibitory effects, thereby promoting and enhancing antitumor immune responses.

2.3. Other immune checkpoints

Apart from the ones mentioned above, there is ongoing research into numerous other immune checkpoints. These novel ICIs have not yet been integrated into the established clinical practices and are presently undergoing evaluation to assess their safety and effectiveness in various cancer types, including melanoma. Notably, inhibitors targeting LAG-3, TIM-3 have progressed to an advanced stage of development [4].

3. ICIs in advanced melanoma

3.1. CTLA-4 inhibitors

A significant breakthrough in its treatment came with Ipilimumab, an antibody against CLTA-4. This drug marked the first instance of an increase in survival rates among melanoma patients [5]. 676 patients with advanced melanoma who had previously received treatment participated in the first phase III study and were randomly divided into three groups with a 3:1:1 ratio: an ipilimumab monotherapy arm (at the dose of 3 mg/kg), a gp-100 peptide vaccine arm, or a combination arm. Every 3 weeks, each agent was given out four times. As compared to the vaccination alone, the data demonstrate that ipilimumab significantly enhanced median overall survival (OS) by roughly 3.6 months in both ipilimumab arms. More impressive benefit was the effect of ipilimumab in 2 years survival, 18% compared with 5% with vaccine alone. There were not that many differences found between two ipilimumab groups.

Tremelimumab was previously considered a potential treatment for advanced melanoma. Nevertheless, it did not exhibit a survival advantage compared to chemotherapy in a phase III trial, and as a result, it has not been currently used for this purpose [6].

An essential characteristic that sets CTLA-4 inhibitors apart from conventional chemotherapeutic agents and other drugs is their response rate. Unlike chemotherapies, which typically yield responses

within a few weeks after treatment initiation, responses to ICIs can be considerably slower, sometimes taking several months to manifest. This disparity demands reassessment of response criteria, away from those originally developed based on experiences with chemotherapeutic agents.

3.2. PD-1 inhibitors

In June and July 2015, two PD-1 inhibitors, nivolumab and pembrolizumab, received approval for the therapy of advanced melanoma as well. Unlike ipilimumab, which works mainly in the on-phase phase, PD-1 inhibitors work by inhibiting negative regulation of T cells during the reactive phase. As a fully human IgG4 mAb, in a phase 3 clinical study of melanoma, nivolumab administered 3 mg/kg every two weeks was compared with chemotherapy as the doctor's choice [7]. Nivolumab had significantly higher objective response rates (ORR) and median OS than other treatments, at 31.7% versus 10.6%, 16 versus 14 months.

Pembrolizumab is a humanized IgG4 mAb. Its clinical trials showed that compared experiments with doses of 2 or 10 mg / kg every 3 weeks, it was possible to obtain a prolonged PFS in patients [8]. Interestingly, in a comparative study between pembrolizumab and ipilimumab at appropriate doses, pembrolizumab showed higher PFS (6-months: 46,4% vs. 26,5%) and OS (1 year survival: 68% vs. 58%) [9]. An extended follow-up verified the outcome. Antibodies against PD-1 are used much more frequently in the clinic than those against CTLA-4 over time due to stronger clinical efficacy and generally better tolerability.

4. ICIs combination therapy

ICIs have developed promising in human malignancies. But for better treatment and to avoid drug resistance and deficiencies in immunotherapy, drugs are combined therapy. A large number of experimental results show that ICI will be safe and effective with conventional anti-cancer therapy, targeted molecular compounds and new immunomodulatory therapies. Current CTLA-4 and PD-1 inhibitors reduces morbidity in patients.

4.1. With conventional therapy

In one experiment, more than five hundred patients diagnosed with untreated metastatic melanoma were randomized to divide the patients into two groups. The first group received ipilimumab (10 mg/kg) and dacarbazine (850 mg/m²). The second group received dacarbazine and placebo. These treatments were administered in cycles at weeks 1, 4, 7, and 10 [10]. Subsequently, dacarbazine alone was administered to patients every two weeks until week 22. The results showed that the OS was 1.9 months longer in the group receiving ipilimumab and dacarbazine than in the other group. Additionally, the ipilimumab group exhibited higher survival rates at various time intervals, including 1 year (47.3% vs. 36.3%), 2 years (28.5% vs. 17.9%), and 3 years (20.8% vs. 12.2%).

Besides dacarbazine, there are also clinical data shows radiotherapy may potentiate anti-melanoma activity of ICI. Several recent studies demonstrated prolonged response and survival when radiotherapy combined with ICI. This will hopefully give new therapeutic insights for advanced melanoma patients.

4.2. With other ICIs

In combination therapy using Ipilimumab and Nivolumab, the immune response's synergistic efficacy against metastatic melanoma has surpassed the usage of monotherapy. In a double-blind study, over a hundred patients with untreated metastatic melanoma, with 87% of them having advanced disease, were allocated in a 2:1 ratio. They either received a combination of ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) or a placebo. These treatments were given four times with nivolumab or placebo every three weeks until disease progression or inability to be exposed to toxic effects [11]. The findings showed that the combination group had a higher rate of objective response (61% vs. 11% in the placebo group), and more frequent complete response was observed in 22%

among combination group, while no patients in placebo group. The combination group showed more favourable results, with a 68 percent reduction in median change in tumor volume assessed by the investigators, compared with a 5.5 percent increase in the other group. There was also a 6.5-year analysis that revealed the results of combination therapy in patients with advanced melanoma [12]. The median OS of patients treated with ipilimumab and nivolumab was 72.1 months, which was superior to nivolumab alone, 36.9 months for nivolumab and 19.9 months for ipilimumab alone.

Moreover, there is another promising combination, which is relatlimab (LAG-3 inhibitor) and nivolumab, known as opdivo. In NCT03470922 experiment, more than 700 untreated melanoma patients were randomly assigned to receive nivolumab (480 mg/kg) plus placebo every four weeks or the same dose of nivolumab plus relatlimab (160 mg/kg). When relatlimab was added, the result showed a median PFS of 5.5 months that was longer than the placebo's. It was the first FDA-Approved immunotherapy to target LAG-3 for advanced melanoma treatment.

4.3. With traditional Chinese medicine (TCM)

Due to the large number of Chinese and the high incidence of cancer, traditional Chinese medicine was developed to treat cancer. Previous studies have shown that TCM can inhibit the emergence of cancer by inhibiting tumor cells, and this treatment has also achieved good results. In TCM, yin and yang balance are used to treat each ailment, which is also equivalent to boosting immunity to maintain balance. More and more research directions combine TCM with ICI to improve the efficacy of ICI and treat irAE [13]. In the development of Chinese medicine, human cancer occurs because of the internal causes of the human body and the external causes of the environment, one because of the seven emotions, and the other because of the internal injuries caused by the seven immoralities of the environment. In previous studies, it has been found that traditional Chinese medicine can fight tumors and greatly improve immunotherapy, improve drug resistance, reduce the clinical manifestations of related diseases and prolong the life span. For example, drugs ginseng and skullcap will enhance innate and adaptive immunity, as well as Haier particles and icariin in the finished drugs, which are one of the drugs to treat tumors. In the treatment of PD-1 tumors, TCM can play an inhibitory role. The inhibition method is to combine PD-1/PD-L1 interaction so that it will not lead to tumor immunity escape. Berberine in proprietary Chinese medicines has deubiquitinating activity, so it can be used to ubiquitinate and degrade PD-L1 to inhibit the axis, thereby reducing the cancer rate. The research report of Zhang et al. proved that Compound Chinese medicine CFF-1 can inhibit the expression of PD-1 in prostate cancer cells by adding or subtracting doses with time and dose [14]. However, TCM is still used less in the treatment of ICI and there are few experimental data, so it still needs to be confirmed by a large number of experiments and comprehensively added to the treatment to better play a better role.

5. Toxicities and immune-related Adverse Event (irAEs)

While ICIs provide enhanced survival benefits, they may also cause irAEs due to questions about their drug principles. Ways to inhibit the modulated immune response are that ICI stimulates the immune system and can lead to inflammation of various organs, thereby increasing the risk of irAEs. Multiple tissues and systems are involved in many adverse reactions, including the skin, gastrointestinal tract, endocrine system, etc. ICI treatment is associated with increased treatment of pruritus and diarrhoea compared with conventional chemotherapy regimens. The incidence of irAE with combination therapy was notably better than that in monotherapy, which increased the focus of experimental data.

To reduce toxicity while maintaining efficacy, nivolumab every 2 weeks and pembrolizumab once every 3 weeks are the agents with the lowest risk of irAE in patients. Ipilimumab is administered intravenously, 12 axes divided into every 3 weeks [15]. In addition, studies to date have shown that atezolizumab is one of the most favourable ICI and is extremely low relative to irAE risk in various cancer types. However, it has yet to be approved by the FDA as an advanced melanoma treatment

option. Additional studies were conducted as well. Tocilizumab was used experimentally, adding IL-6 receptor blocking antibodies in combination with ipilimumab and nivolumab. The experimental results showed that the higher prognosis of melanoma IL-6 was associated with the symptoms of irAEs during ICI treatment [16]. ICI is very common in combination therapy for melanoma and in BRAF V600 mutations. Sensitivity to BRAF and MEK inhibitors was detected in some patients, in half of melanoma tumors. Thus, new combinations of targeted drug therapies have been made, representing the basis of BRAF mutant melanin therapy.

Although first-line treatment for advanced melanochroma is already ICI, physicians need to tailor treatment plans to optimize treatment outcomes while minimizing adverse events that may lead to treatment interruption or ineffectiveness. In particular, ICI is recommended for patients with pre-existing autoimmune disease to prevent such complications if any serious irAE is initiated. In fact, studies have shown that patients with irAEs get good outcomes in melanoma treatment during ICI treatment. But to date, it is unclear with the correlation between specific irAEs occurrence and ICI efficacy in melanoma yet.

6. Resisances

Beyond irAEs, another factor that significantly impacts the clinical outcomes of ICI is the development of resistance. Patients who do not experience any therapeutic benefit are typically classified as having innate ICI resistance, while there is another group of patients who initially respond positively but then lose their clinical benefit, characterized by acquired ICI resistance. These resistance phenomena can be attributed to both tumor-intrinsic and tumor-extrinsic mechanisms [17].

In the case of tumor-intrinsic resistance, cells undergo modifications in processes related to cell signalling pathways, DNA damage response, and immune recognition. Conversely, mediated signals between the external environment of the tumor and various immune cells are key to the tumor's extrinsic drug resistance. This type of drug resistance tends to emerge after initial clinical treatment and then during tumor treatment. It affects the inheritance of tumor cells presenting tumor neoantigens and the regulation of gene expression, and changes DNA and proteins on chromosomes through chemical modifications, thereby affecting gene expression. are key triggers of both drug resistance.

The relatively low overall response rate of ICI in melanoma patients can be attributed to these resistances. However, there are many studies that have attempted to determine the biomarkers of ICI in melanoma patients and thus predict which type of treatment will result from the best treatment. One such biomarker is TMB, which represents the count of mutations in the DNA of somatic cells within large tumors. High TMB is closely related to the better response of patients during treatment. However, it is not widely recognized as an effective treatment option for ICI melanoma, as a large proportion of melanoma patients exhibit high TMB, but not all patients derive clinical benefit from ICI treatment. Besides TMB, PD-L1 expression, and even the gut microbiome has shown correlations with ICI response [18]. For now, none of these factors has been well established as a reliable treatment specimen for predicting ICI response in melanoma patients.

7. Future direction and emerging therapies

Although ICIs of CTLA-4 and PD-1 in targeted therapies have greatly transformed the cancer treatment and improved cure rates in a large number of research results, there is still an urgent need for new treatment options. Novel and promising anti-cancer regimens have been developed in congenital ICI that can be used alone, with anti-cancer antibodies, and with adaptive ICI. In TCM treatment, because there are also fewer experimental data, there is no more or more effective experimental data for combination therapy to promote the treatment of cancer, and a large number of experimental data should be carried out in TCM to play a role. In the study, Drugs that can activate the adaptive immune system by increasing antibody and antigen presentation, or that can alter the innate immune system's response to tumors, may increase the rate at which tumors are successfully

treated. These drugs also exert phagocytosis and natural cytotoxicity. There will be a large amount of overlapping data in the treatment data, and the regulation and expression of checkpoints between innate immunity and adaptive immunity should be rationally allocated. The use of new gene screening techniques is that other surface regulators of innate immune cell function can serve as potential therapeutic targets. Innate ICIs are an innovative novel medication for the treatment of both solid and hematologic malignancies that has the potential to increase the use of immunotherapy in many different cancer types and considerably improve patient outcomes, especially melanoma.

8. Conclusion

Over the past few years, advanced melanoma treatment has undergone a revolution thanks to immunotherapy based on ICI. Multiple studies have demonstrated rapid improvements in applications of ICIs such as CTLA-4 and PD-1 as either monotherapy or combination with other chemotherapeutic or immunotherapeutic drugs or TCM based on meeting strict security and efficacy standards. Therefore, the sample size can be further expanded and the evidence-based evidence can be improved. However, challenges as toxicities and immune-related adverse effects, as well as resistances to ICIs remain. The fact that ICIs will only be beneficial for a small percentage of patients and that they are likely to be expensive, it is essential to estimate predictive biomarkers. Therefore, focus on patients who is most likely to take advantage of ICI treatment. Currently, there are several novel ICIs and novel biomarkers under investigation. All of these efforts will result in a deeper understanding of the mechanisms of ICI, thus facilitating further development, providing hope for more patients with advanced melanoma.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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