

Immune checkpoint inhibitors: the mechanisms, limitations, and improvements

Fangting Zhang*

Depu Foreign Language School, Chongqing, China

*Corresponding author: 2021001682@poers.edu.pl

Abstract. Cancer has been a huge public health concern for decades, with a high incidence and death rate. Traditional therapeutic methods are not effective enough, with many side effects. While immune checkpoint inhibitors, as immunotherapy, are thought to have the most promising future development, aiming at activating immunity against tumor cells for treatment. They worked by blocking immune checkpoints, for example, CTLA-4 and PD-1. These immune checkpoints control T cells expansion, terminate T-cell responses, thus are responsible for the evasion of cancer cells from the immune system, and the immune responses are suppressed and autoimmunity is prevented by them. Nevertheless, the optimal duration of treatment and resistance to it has not been fully understood. Furthermore, some patients receiving immune checkpoint inhibitor therapy even suffer from immune-related adverse events. Fortunately, previous studies have stated several improvement aspects, including immune-modulatory medications to deal with immune-related adverse events, predictive biomarkers to judge whether the immune checkpoint inhibitors planned to use are more beneficial rather than harmful and monitor the response to treatment, as well as combination therapies, including the application of both two immune checkpoint inhibitors simultaneously, also the use of them in conjunction with molecular targeted therapy. This paper briefly introduced the mechanisms and effects of different checkpoints, for obtaining a clearer understanding of the use of immune checkpoint inhibitors.

Keywords: Immune Checkpoint Inhibitors, Cytotoxic T-lymphocyte-associated Antigen 4 (CTLA-4), Programmed Death 1 (PD-1)/ Programmed Death Ligand 1 (PD-L1), Mechanisms, Limitations.

1. Introduction

The second leading cause of mortality all over the globe, cancer, accounts for approximately 8.2 million deaths out of 14.1 million cases annually. Moreover, it is anticipated that there would be 1.9 million additionally-diagnosed cases of cancer in the United States during 2022. Besides these large cancer-incidence numbers, it has been suggested that every person has a risk to develop cancer [1]. Fortunately, there have been several treatments available for cancer, including immunotherapy, which is a method for combating cancer cells through the strengthened immune system's ability. After the growth of cancer cells originating from the mutations of body cells, there are further mutations, which improves evolutionary fitness by increasing the level of genetic diversity, but makes them more likely to be differentiated as non-self from normal cells by the immune system [2]. As a result, antigens from cancer cells could be presented via antigen-presenting cells (APCs), then T cells could destroy cancer cells through multiple mechanisms, like CD8⁺ T cells. For example, cancer cells' plasma membranes can be disrupted by releasing perforin and granzymes, leading to the apoptosis of them.

However, cancer cells could express immune checkpoint molecules on their surface, just like those found on normal cells. In this way, cancer cells could limit immune recognition, suppress the T cells at immune checkpoints, and evade the attack by the immune system. As cell-surface proteins found on natural killer (NK) cells and T cells, immune checkpoints operate as negative regulatory channels and are, among others. They are primarily found to be a key regulator of the immune system, which preserve immune homeostasis and prevent autoimmunity by regulating T cell activation, apoptosis, and providing self-tolerance through the modulation of the kind, intensity, and duration of immune responses. Meanwhile, immune checkpoints are responsible for the evasion of cancer cells in different types of cancer, since they could express those immune checkpoints on their surface, thus limiting immune recognition and suppressing T cells.

Therefore, the checkpoints inhibition would facilitate suppression of anti-tumor response and reactivate immune responses against cancer cells, which leads to tumor shrinkage and a decrease in metastasis. Primarily, immune checkpoint inhibition is aimed at enabling effector T cells to be less inactivated, especially CD8⁺ T cells, as mentioned above, thus improving tumor-specific immune responses. Furthermore, immune checkpoint blockade has the potential to improve the performance of other immunotherapeutic strategies, including molecular targeted therapy, which is discussed later in this review.

This review focused on immune checkpoint inhibitors, and it briefly introduced the mechanisms of some immune checkpoints, discussed the limitations of immune checkpoint inhibitors, including unclear optimal duration of treatment, drug resistance, as well as toxicities, and summarized present and potential ways of improvements for immune checkpoint inhibitors.

2. The mechanisms of immune checkpoints

In the immune system, an immature T cell is originally grown in the thymus and goes through selection pressure. After the deletion of T cells with significant reactivity to self-peptide, others are released into the bloodstream, spleen, and lymphatic organs, where they take part in the circulation, may come into contact with mutated self-proteins in cancer cases. Numerous immune checkpoints control the activation of T cells to prevent autoimmunity, which is called peripheral tolerance. The immune checkpoint pathways cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) are crucial in this process at different phases during the immune response.

2.1. CTLA-4

Since CTLA-4 destroys potential autoreactive T cells at an early point, typically in lymph nodes, it is considered to lead the immune checkpoints. It was first discovered as a delivering inhibitory signal that plays a key role in terminating immune responses. Besides, CTLA-4 is proved to contribute to the prevention of chronic autoimmune inflammation. Because of its constitutive endocytosis from the plasma membrane the intracellular vesicles of FoxP3⁺ regulatory T cells and activated conventional T cells are where CTLA-4 mostly present in. As a result, around 90% of CTLA-4 are intracellular [4].

CTLA-4 and CD28, which are homologous receptors found on the surface of resting human T cells, belong to a family of immunoglobulin-related receptors with the presentation on CD4⁺ and CD8⁺ T cells, that are responsible for mediating symmetrical functions in T-cell immune regulation [5]. CD80 and CD86 (or known as B7-1 and B7-2), demonstrated in figure 1, are ligands shared by CTLA-4 and CD28 that are found on the surface of APCs, resulting in a costimulatory response. In particular, CD28 binds with CD80 with a greater affinity but CD86 with a decreased affinity to provide costimulatory signals amplifying T cell receptor signaling, which induces survival and proliferation of T cells. In contrast, CTLA-4 binds with two ligands with three times the affinity and higher avidity of CD28, competes with it for ligand binding, as well as regulates T cells expansion and activation, thus terminating T-cell responses. Therefore, CTLA-4 performs to be the antagonist of CD28-mediated costimulation [6].

This intimate correlation between CTLA-4 and CD28 indicates the difficulty of understanding the manipulation of one receptor but ignoring the impact on the other. Although the precise mechanisms of the CTLA-4's suppression effect on T-cell responses are not fully understood, this ligand-sharing system creates a rheostat, which is capable of fine-tuning T-cell responses [7].

Take Ipilimumab, the first CTLA-4 checkpoint inhibitor authorized for treating metastatic melanoma according to the FDA in 2011, as an example. It is a recombinant human immunoglobulin G1κ monoclonal antibody, which interacts with CTLA-4 and inhibits its interaction with CD80 or CD86. Ipilimumab molecule touches the front β-sheet of the CTLA-4 molecule, crosses over it, as well as CD80's and CD86's recognition sites, forming a CTLA-4 and ipilimumab complex. This

demonstrates a crucial factor that contributes to the ipilimumab function is the direct steric overlap between ipilimumab and the binding ligands [8].

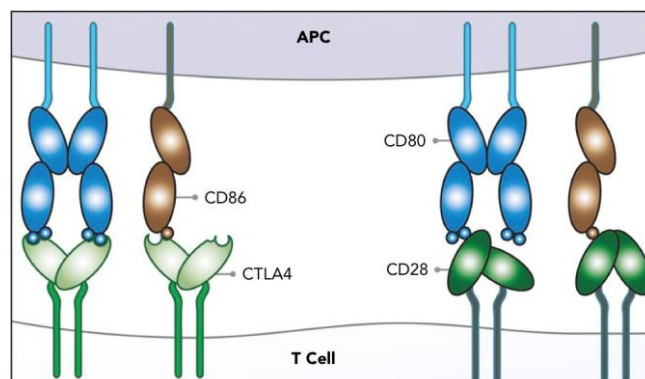


Figure 1. The interactions of CTLA-4 and CD28 with their ligands [7]. For both receptors, CD80 is a dimeric high-affinity ligand, whereas CD86 is a monomeric lower-affinity ligand. CD80 binds to CTLA-4 with greater affinity and avidity than CD86. The relative affinities progress from left to right and from high to low.

2.2. PD-1

While as for PD-1, it is one of the costimulatory receptors in the B7/CD28 family, which is expressed via macrophages, B lymphocytes, monocytes, natural killer cells, activated T cells, and dendritic cells (DCs), especially has a high level of expression on tumor-specific T cells. The PD-1 pathway controls the early-activated T cells later in an immune response, predominantly in peripheral tissues, by binding to programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2), which therefore have wider expression [9]. Specifically, PD-L1 is a transmembrane protein, usually expressed by DCs, some activated T cells, macrophages, epithelial cells, and B cells, particularly under inflammatory conditions, as well as on several types of cancer cells, as an indicator for poorer prognosis [10]. PD-L2, on the other hand, is largely expressed on DCs and monocytes, although on a number of additional immune and nonimmune cells, it may also be triggered, determined by the microenvironment of the host.

In cancer, the activity of PD-1 and its ligands controls anti-tumor immune responses by regulating T cell activation, proliferation, survival, and cytotoxic release, as shown in figure 2. Nearly 30% of solid tumors and hematologic malignancies have been found to apply PD-L1 overexpression for escaping antitumor immune responses, showing the significance of the PD-1/PD-L1 pathway [11]. This pathway, like CTLA-4, is important for immune system balance and preventing autoimmunity. PD-1 interacts with PD-L1, leading to the downregulation, apoptosis, and exclusion from the tumor microenvironment of T cells thus performs as an inhibitor of both innate and adaptive immune responses. In addition, this pathway is responsible for the cytokine secretion inhibition. These allow the escape of cancer cells from the immune system's attack.

To deal with this situation, the inhibition of PD-1/PD-L1 pathway, which mediates antitumor activity and enhances the T-cell response, could be a viable option. For instance, nivolumab, a completely human monoclonal anti-PD-1 antibody, has also gained approval from FDA for treating metastatic melanoma, with quick and durable responses in clinical trials, as well as relatively low adverse events compared to other targeted and immunotherapies [12].

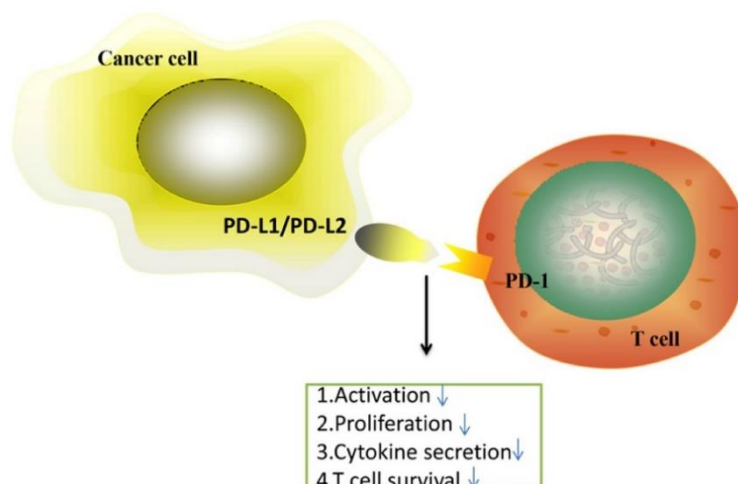


Figure 2. The PD-1/PD-L1 pathway suppresses T cell activation, proliferation, survival, as well as cytotoxic secretion [10].

3. Limitations

As an attractive and promising approach to treat cancer, immune checkpoint inhibitors (ICIs) are rapidly developing, but some limitations are yet to be overcome.

3.1. The optimal duration

The appropriate period of time required for patients to keep receiving ICIs therapy is yet unknown. For patients who have undergone complete responses, they might have a viable option to stop treatment, because durable complete remission has been reported in several studies [13-15]. For example, one of the studies suggests that patients with metastatic melanoma could experience long-term remission following discontinuation of pembrolizumab, an antibody targeting PD-1 [16]. However, more research is required to evaluate the appropriate treatment duration, so that the appropriate time to stop treatment after complete response could be determined, and also to judge whether patients have been over-treated in previous years, which results in physical toxicities, usually involves skin, lung, and endocrine glands, as well as a heavy financial burden on both the patients and the health system.

3.2. Innate and acquired resistance

Resistance triggered by ICIs could be divided into two categories. On the one hand, innate resistance refers to patients who are unable to respond at all but who still develop swiftly or ultimately. Acquired resistance, on the other hand, describes individuals whose early response to ICI treatment could be induced for a length of time, but then experience clinical or radiological disease progression. At present, the mechanisms behind innate and acquired resistance towards this treatment still remain unknown. To frame the discussion around this notion, the mechanisms of action of ICIs must be investigated in order to identify the immune system's suppressive influence on tumor formation.

It is generally suggested that in the tumor microenvironment (TME), the antigen-experienced T cells must be reactivated and clonal-proliferated for mounting an anti-tumor immune response. [17,18]. First of all, tumor-related antigens being processed and presented accurately through antigen-presenting cells, as well as recognition of these displayed antigens, are required for the production of tumor-reactive T lymphocytes. A specific T-cell receptor would achieve this, enables the T-cell activation. After that, effector T cells could be differentiated from the tumor-specific T cells, experience clonal expansion and then pass to the TME, aimed at destroying cancer cells. To generate long-term immunity, a fraction of effector T cells have to develop into effector memory T cells (TEM).

As a result, failure in any of the steps mentioned above would result in an unsuccessful ICI therapy, and these could be simply classified into three reasons: deficient generation of anti-tumor T cells, weakened function of tumor-specific T cells, and damaged formation of T-cell memory [19].

3.3. Immune-related adverse events and toxicities

It is suggested that ICIs could only benefit a fraction of cancer patients, whereas some of the patients receiving ICI therapy have to suffer from immune-related adverse events (irAEs). Inhibiting immune checkpoints which strengthen the body's natural defenses against autoimmune, results in different kinds of local and systemic autoimmune responses, so that irAEs occurs in consequence. At first, irAEs tend to be organ-specific, instead of affecting different organs at the same time. After that, the toxicities onset is regularly delayed. In detail, they would be delayed until the fourth week and tenth week when using ipilimumab and nivolumab respectively [20]. Although these side effects are generally manageable, which means they could be migrated by dose modification, administration of steroids, and discontinuation of therapy, they might also be fatal in some cases and are inevitable by dose reductions, sometimes even trigger permanent damage to patients.

Interestingly, irAEs seem to be correlated to the rates of clinical response [21,22]. More specifically, anti-PD-1 and anti-PD-L1 antibody responses are thought to be more closely linked to irAEs than anti-CTLA-4 antibody responses. The underlying association between irAE features (location, severity, onset time, and management) and ICI therapeutic effectiveness is, however, unknown.

4. Improvements

Even though ICI therapies have limitations, including the undetermined optimal duration, innate and acquired resistance, as well as various irAEs and toxicities, there are several ways of present and potential improvements.

4.1. Immune-modulatory medications

The irAEs induced by ICI therapy are generally managed with immune-modulatory medications (IMM), as shown in Table 1. Among them, steroids are the most frequent first-line therapy for most non-endocrine irAEs, given when the severity of the irAEs justifies inflammation reversal. Some patients, however, are unresponsive to steroids or develop resistance to them. There is minimal evidence to guide their care, but several additional options, such as infliximab and mycophenolate mofetil, are available [23,24].

Table 1. The mechanisms of action of immune-modulatory medications [23].

Drug	Crucial mechanism
Steroids	Inhibits interleukin transcription and neutrophil apoptosis, decreases cytokine manufacturing, and diminishes macrophage activity, results in multiple impacts on B cells, T cells, and phagocytes.
Mycophenolate mofetil	Inhibits the enzyme named inosine monophosphate dehydrogenase (IMPDH), which plays a role in nucleotide production, especially in activated lymphocytes.
Infliximab	An antibody to stop the inflammatory cytokine tumor necrosis factor alpha (TNF- α) from binding to its receptors.
Tacrolimus and cyclosporine	A calcineurin inhibitors involving in T cell proliferation, suppress the transcription of interleukin 2 (IL-2).

4.2. Biomarkers

Since not all of the patients would benefit from the ICI therapies, predictive biomarkers are required. Biomarkers could be utilized to differentiate responders and non-responders, thus are able

to determine whether, or which ICI therapies are appropriate for a particular patient, as well as monitor the response to therapy, aiming at reducing irAEs and improving treatment outcome.

There have been plenty of searches for predictive biomarkers, which result in PD-L1 overexpression, genetic signatures, neoantigens, immune cells, epigenetic signatures being promising predictors, and various additional markers are now being researched [25-28].

Take PD-L1 overexpression as an example. In general, the immune-inflamed phenotype is related to anti-PD-1/PD-L1 therapy, and due to the adaptive expression of effector T cells via most cell types after exposure to IFN- γ , PD-L1 could indicate the activity of them. Therefore, firstly identifying the presence of PD-L1 expression in tumor tissues provides a straightforward means of assessing the efficacy of PD-1/PD-L1 treatment. Type I tumor microenvironments are defined as PD-L1-positive tumors with tumor-infiltrating lymphocytes and are suggested to react best to immune checkpoint inhibition. Moreover, it is proved in a clinical trial of pembrolizumab for treating non-small cell lung carcinoma that in more than 50% of tumor cells, patients with PD-L1 expression have higher overall survival and survival with no signs of relapse [29].

While as for genetic signatures, a group of patients suffering from breast cancer, who had a recurrence of the tumor after 1 to 5 years compared to a 7-year relapse-free survival has been studied, and over 299 immune-related genes are screened, then eventually found that five genes, including occludin (OCLN), guanylate binding protein 1 (GBP1), immunoglobulin kappa chain locus (IGKC), immunoglobulin lambda-like polypeptide 5 (IGLL5), and signal transducer and activator of transcription 1 (STAT1), were considerably overexpressed in patients who had no relapses [30], so that they have the potential to become predictive biomarkers (figure 3).

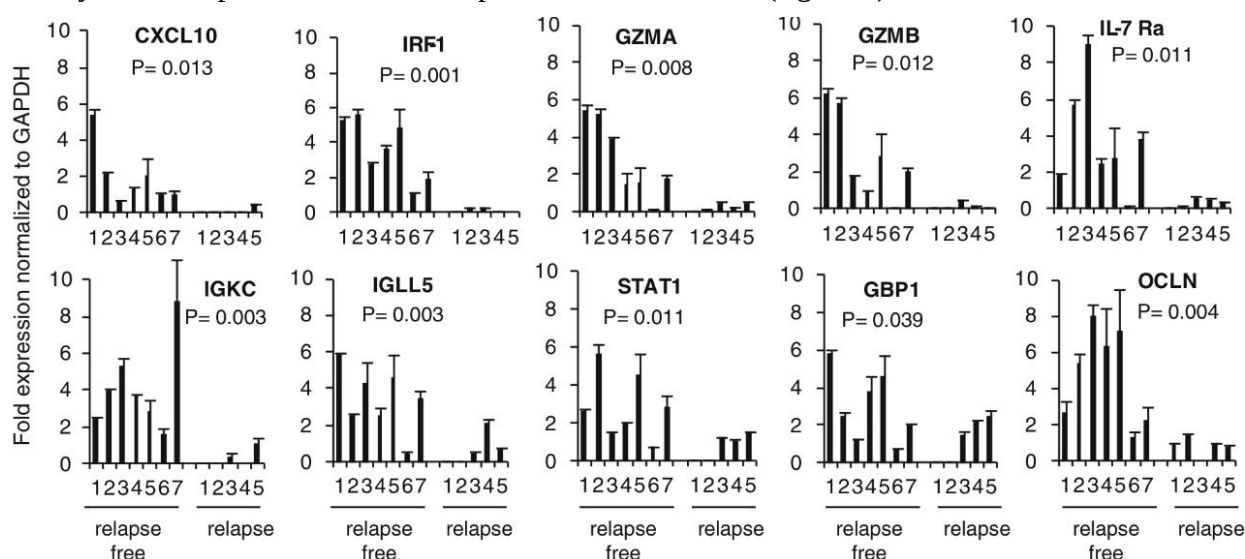


Figure 3. Real-time PCR analysis of relapse-free versus relapse patients' frozen tumor tissues [30].

The validation group consisted of two cohorts of patients whose malignancies were exposed to confirmatory real-time PCR. After normalization to GAPDH, the data are shown as the average mean of triplicate wells.

4.3. Combination therapies

Because of the enhanced compensatory expression of the PD-1/PD-L1 pathway, ipilimumab monotherapy is inadequate to trigger an anti-tumor response in most cancer cases. To enhance efficacy and overcome the resistance of ICI therapies, the combination therapies, which is the application of different antibodies against two immune checkpoints at the same time, are studied. For example, the simultaneous use of CTLA-4 and PD-1 inhibitors, ipilimumab and nivolumab respectively, gain great progress in the therapies to metastatic melanoma, renal cell carcinoma, non-small cell lung cancer, and many different malignancies. Specifically, patients suffering from metastatic melanoma have been shown to have a much higher response rate and enhanced survival. However, combination therapies carry a greater risk for adverse events, such as headache, nausea,

and hypothyroidism. In consequence, different dosing strategies are applied, for instance, 3mg/kg ipilimumab plus 1mg/kg nivolumab in melanoma, whereas 1mg/kg ipilimumab plus 3mg/kg nivolumab in renal cell carcinoma [31]. The reason for these careful dose selections is to balance efficacy and toxicity.

Due to the relatively low response rate of ICIs in common cancer types and the resistance to them, it is also possible to improve the treatment by combining immunotherapy with molecular targeted therapy. The underlying processes of tumor relapse have recently been correlated to faulty regulation of critical molecules in immune and cancer cells, highlighting the need for this kind of individualized combination therapy based on specific molecular abnormalities. For instance, targeting the Wnt/ β -catenin signaling pathway, which is among the most well-studied cancer drivers, is expected to be added to cancer immunotherapy because it promotes cancer progression through controlling the tumor-immune cycle in most nodes, including DCs, T cells, and cancer cells. Thus, this method is suggested to be able to overcome the main types of resistance through various mechanisms [32], shown in Table 2 in detail.

Table 2. The mechanisms underlying the different types of resistance overcome by targeting Wnt/ β -catenin signaling [32].

Type	Mechanism
Primary resistance	Damaged antigen presentation
	Absence of tumor antigen
	Genetic T cell exclusion
	Antigen-specific T cells in short supply
Adaptive resistance	Changes in the antigen-presenting apparatus
	Regulatory immune cells activation
	Immune checkpoints expression
	No response to T cells
Acquired resistance	Destroyed T cell activation
	Weakened recognition of tumor antigen
	Escape variant development

5. Conclusion

The main hurdles to eliciting anti-tumor immune responses are considered to be the immune checkpoints. The dysfunction of effector T-cell response is triggered by the activity of them, like the most commonly researched CTLA-4 and PD-1. Similarly, due to the suppressive effects of immune checkpoints, other anti-tumor immunotherapies have not been successful in eliciting long-term responses, demonstrating how significant ICIs are in improving therapeutic efficacy. Also, considering the lack of effective therapy for cancer, compared to conventional therapies, ICIs could benefit some cancer patients and achieve a significant increase in their survival. However, many limitations still need to be solved, including not only unclear optimal duration, innate and acquired resistance to the treatment, but also immune-related adverse events and toxicities to patients, several improvement methods have been pointed out, such as immune-modulatory medications, predictive biomarkers, and combination therapies. As a method for treating cancer by activating antitumor immunity, ICIs, the most studied type of immunotherapy with promising possibilities of development, have the potential to enhance treatment results for cancer patients, but the mechanisms behind them need to be more deeply understood in the future.

References

- [1] Atlanta, G. A. (2022). American Cancer Society. Cancer facts & figures 2022. American Cancer Society.
- [2] Chen, D. S., & Mellman, I. (2017). Elements of cancer immunity and the cancer-immune set point. *Nature*, 541(7637), 321-330.

- [3] Dyck, L., & Mills, K. H. (2017). Immune checkpoints and their inhibition in cancer and infectious diseases. *European journal of immunology*, 47(5), 765-779.
- [4] Shiratori, T., Miyatake, S., Ohno, H., Nakaseko, C., Isono, K., Bonifacio, J. S., & Saito, T. (1997). Tyrosine phosphorylation controls internalization of CTLA-4 by regulating its interaction with clathrin-associated adaptor complex AP-2. *Immunity*, 6(5), 583-589.
- [5] Hosseini, A., Gharibi, T., Marofi, F., Babaloo, Z., & Baradaran, B. (2020). CTLA-4: From mechanism to autoimmune therapy. *International Immunopharmacology*, 80, 106221.
- [6] Lingel, H., & Brunner-Weinzierl, M. C. (2019, April). CTLA-4 (CD152): A versatile receptor for immune-based therapy. In *Seminars in immunology* (Vol. 42, p. 101298). Academic Press.
- [7] Rowshanravan, B., Halliday, N., & Sansom, D. M. (2018). CTLA-4: a moving target in immunotherapy. *Blood, The Journal of the American Society of Hematology*, 131(1), 58-67.
- [8] Ramagopal, U. A., Liu, W., Garrett-Thomson, S. C., Bonanno, J. B., Yan, Q., Srinivasan, M., ... & Almo, S. C. (2017). Structural basis for cancer immunotherapy by the first-in-class checkpoint inhibitor ipilimumab. *Proceedings of the National Academy of Sciences*, 114(21), E4223-E4232.
- [9] Chen, D. S., Irving, B. A., & Hodi, F. S. (2012). Molecular pathways: next-generation immunotherapy— inhibiting programmed death-ligand 1 and programmed death-1. *Clinical cancer research*, 18(24), 6580-6587.
- [10] Han, Y., Liu, D., & Li, L. (2020). PD-1/PD-L1 pathway: current researches in cancer. *American journal of cancer research*, 10(3), 727.
- [11] Dermeni, F. K., Samadi, P., Rahmani, G., Kohlan, A. K., & Najafi, R. (2019). PD-1/PD-L1 immune checkpoint: potential target for cancer therapy. *Journal of cellular physiology*, 234(2), 1313-1325.
- [12] Koppolu, V., & Vasigala, V. K. R. (2018). Checkpoint immunotherapy by nivolumab for treatment of metastatic melanoma. *Journal of cancer research and therapeutics*, 14(6), 1167.
- [13] Tan, A. C., Emmett, L., Lo, S., Liu, V., Kapoor, R., Carlino, M. S., ... & Menzies, A. M. (2018). FDG-PET response and outcome from anti-PD-1 therapy in metastatic melanoma. *Annals of Oncology*, 29(10), 2115-2120.
- [14] Jansen, Y. J. L., Rozeman, E. A., Mason, R., Goldinger, S. M., Foppen, M. G., Hojberg, L., ... & Neyns, B. (2019). Discontinuation of anti-PD-1 antibody therapy in the absence of disease progression or treatment limiting toxicity: clinical outcomes in advanced melanoma. *Annals of Oncology*, 30(7), 1154-1161.
- [15] de Miguel, M., & Calvo, E. (2020). Clinical challenges of immune checkpoint inhibitors. *Cancer Cell*, 38(3), 326-333.
- [16] Robert, C., Ribas, A., Hamid, O., Daud, A., Wolchok, J. D., Joshua, A. M., ... & Hodi, F. S. (2018). Durable complete response after discontinuation of pembrolizumab in patients with metastatic melanoma. *Journal of clinical oncology*, 36(17), 1668-1674.
- [17] O'Donnell, J. S., Long, G. V., Scolyer, R. A., Teng, M. W., & Smyth, M. J. (2017). Resistance to PD1/PDL1 checkpoint inhibition. *Cancer treatment reviews*, 52, 71-81.
- [18] Sharma, P., Hu-Lieskovan, S., Wargo, J. A., & Ribas, A. (2017). Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*, 168(4), 707-723.
- [19] Jenkins, R. W., Barbie, D. A., & Flaherty, K. T. (2018). Mechanisms of resistance to immune checkpoint inhibitors. *British journal of cancer*, 118(1), 9-16.
- [20] Andrews, M. C., Duong, C. P., Gopalakrishnan, V., Iebba, V., Chen, W. S., Derosa, L., ... & Wargo, J. A. (2021). Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade. *Nature medicine*, 27(8), 1432-1441.
- [21] Das, S., & Johnson, D. B. (2019). Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. *Journal for immunotherapy of cancer*, 7(1), 1-11.
- [22] Malnick, S. D., Abdullah, A., & Neuman, M. G. (2021). Checkpoint inhibitors and hepatotoxicity. *Biomedicines*, 9(2), 101.
- [23] Spain, L., Diem, S., & Larkin, J. (2016). Management of toxicities of immune checkpoint inhibitors. *Cancer treatment reviews*, 44, 51-60.

- [24] Beattie, J., Rizvi, H., Fuentes, P., Luo, J., Schoenfeld, A., Lin, I. H., ... & Hellmann, M. D. (2021). Success and failure of additional immune modulators in steroid-refractory/resistant pneumonitis related to immune checkpoint blockade. *Journal for immunotherapy of cancer*, 9(2).
- [25] Gandhi, L., Rodríguez-Abreu, D., Gadgeel, S., Esteban, E., Felip, E., De Angelis, F., ... & Garassino, M. C. (2018). Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. *New England journal of medicine*, 378(22), 2078-2092.
- [26] Schmid, P., Adams, S., Rugo, H. S., Schneeweiss, A., Barrios, C. H., Iwata, H., ... & Emens, L. A. (2018). Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *New England Journal of Medicine*, 379(22), 2108-2121.
- [27] Motzer, R. J., Penkov, K., Haanen, J., Rini, B., Albiges, L., Campbell, M. T., ... & Choueiri, T. K. (2019). Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *New England Journal of Medicine*, 380(12), 1103-1115.
- [28] Tabernero, J., Van Cutsem, E., Bang, Y. J., Fuchs, C. S., Wyrwicz, L., Lee, K. W., ... & Shitara, K. (2019). Pembrolizumab with or without chemotherapy versus chemotherapy for advanced gastric or gastroesophageal junction (G/GEJ) adenocarcinoma: The phase III KEYNOTE-062 study.
- [29] Reck, M., Rodríguez-Abreu, D., Robinson, A. G., Hui, R., Csőszi, T., Fülöp, A., ... & Brahmer, J. R. (2016). Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. *N engl J med*, 375, 1823-1833.
- [30] Ascierto, M. L., Kmiecik, M., Idowu, M. O., Manjili, R., Zhao, Y., Grimes, M., ... & Manjili, M. H. (2012). A signature of immune function genes associated with recurrence-free survival in breast cancer patients. *Breast cancer research and treatment*, 131(3), 871-880.
- [31] Sharma, P., Pachynski, R. K., Narayan, V., Fléchon, A., Gravis, G., Galsky, M. D., ... & Fizazi, K. (2020). Nivolumab plus ipilimumab for metastatic castration-resistant prostate cancer: preliminary analysis of patients in the CheckMate 650 trial. *Cancer cell*, 38(4), 489-499.
- [32] Wang, B., Tian, T., Kalland, K. H., Ke, X., & Qu, Y. (2018). Targeting Wnt/ β -catenin signaling for cancer immunotherapy. *Trends in pharmacological sciences*, 39(7), 648-658.