

The role of PD-1/PD-L1 inhibitors in the treatment for non-small cell lung cancer (NSCLC)

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Abstract. Non-small cell lung cancer (NSCLC) is a frequent cancer that affects people around the world. Checkpoint inhibitors are the most intensively studied treatment methods at present. Activation of PD-1/PD-L1 is accountable for suppressing the activation, proliferation, and cytotoxic secretion of T cells. By giving PD-1 /PD-L1 inhibitors, an individual's immune system can be boosted to some extent. Nivolumab, pembrolizumab, atezolizumab, and durvalumab have shown to be effective for NSCLC patients in clinical trials. The effectiveness of PD-1/PD-L1 inhibitors combining with SBRT, chemotherapy, or other checkpoint inhibitors to treat NSCLC has also been demonstrated. However, PD-1/PD-L1 inhibitors also have drawbacks, such as non-specific recognition misses, and immune-related adverse events. This article mainly discusses the role of PD-1/PD-L1 inhibitors in the treatment for NSCLC.

Keywords: PD-1/PD-L1 Inhibitor, Non-small Cell Lung Cancer (NSCLC), Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab.

1. Introduction

Cancer has been a huge public health concern for decades. In a 2019 report, approximately 1.76 million new cases were diagnosed and 606 thousand deaths due to cancer in America, 22% of deaths in America is due to cancer in 2016 [1]. Non-small cell lung cancer (NSCLC) is responsible for roughly 85% of lung cancer cases [2]. The lung is a complicated yet delicate organ. This complex community of cells can experience a cascade of cell-autonomous and microenvironmental changes that shift the balance of cellular division and death; thus allowing immune recognition to be avoided, ultimately leading to the cancer.

NSCLC has a relatively high somatic tumor mutation burden (TMB), especially in smokers, who account for a big proportion. The treatment of lung cancer has evolved from the empirical use of cytotoxic drugs to personalized medicine. Tumors must have genetic changes that prevent them from an effective immune response. Many tumors invalidate the immune system by expressing certain molecules, such as the programmed death 1 and its ligand (PD-1 and PD-L1), which are implicated in the T cell killing [3]. PD-L1, which binds to PD-1 and so inactivates PD-1-expressing T cells, is increased in several NSCLCs.

Therefore, immunotherapy is an approach to kill cancer cells by enhancing the immune system. To evade the immune response, tumors rely on a range of immunosuppressive mechanisms that are complementary to one another and many of which function simultaneously. It is possible that different mediators have a role in inhibiting dendritic cells (DCs) by either reducing T-cells that penetrate into tumor bed or decreasing the activation of effector T-cells and simultaneously promoting Treg cells to become more active [4]. Figure 1 shows some different mechanisms of immunosuppression, such as checkpoint PD-1 which inhibit the activity of T-cells. There are various immunotherapies developed based on immunosuppression, some options are checkpoint inhibitors, cancer vaccines, cytokines, CAR T-cell, monoclonal antibodies, etc.

Immune checkpoints regulate costimulatory and co-inhibitory signals, which is vital in maintaining self-tolerance as well as the magnitude and duration of T-cell responses. The interplay

between PD-1 and PD-L1 or PD-L2 leads to signal transduction that suppresses T-cell proliferation, cytokine generation, and cytolytic activity. PD-L1 is a transmembrane protein which is thought to be a co-inhibitory component in the immune response [5]. When combined with PD-1, it has the ability to limit cell proliferation, block their cytokine release, and trigger apoptosis in the affected cells. This paper introduces the mechanism of PD-1 and PD-L1 and discusses the usage of PD-1/PD-L1 inhibitors in NSCLC patients.

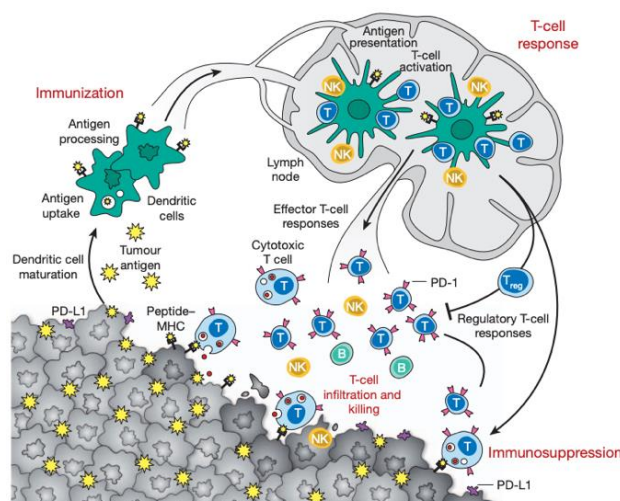


Figure 1. Regulation in anti-tumor immunity [4].

2. The discovery of checkpoints

The earliest prototype of immunotherapy data dates back to 1891, when a surgeon in New York, William Coley, began injecting inactivated or live streptococcus pyogenes and Serratia Marcescens into tumors. The stimulations of the immune system by the infection eventually show some positive results in the elimination of the tumor in the patient. In 1992, the discovery of PD-1, an immune checkpoint that is now widely recognized, was made by Ishida et al.

3. The mechanism of PD-1 / PD-L1 and their inhibitors

3.1. The pathway of PD-1 / PD-L1

In many malignancies, PD-L1 plays a key part in immunotherapy by suppressing the host's responses to tumor cells. The protein PD-L1 is produced by parts of macrophages on a constitutive basis, but it has been shown to be quickly increased in a variety of different tissue types and by malignancies in response to interferon-gamma or other inflammatory mediators. Tolerance to peripheral substances is maintained by the action of PD-1 and its ligands together.

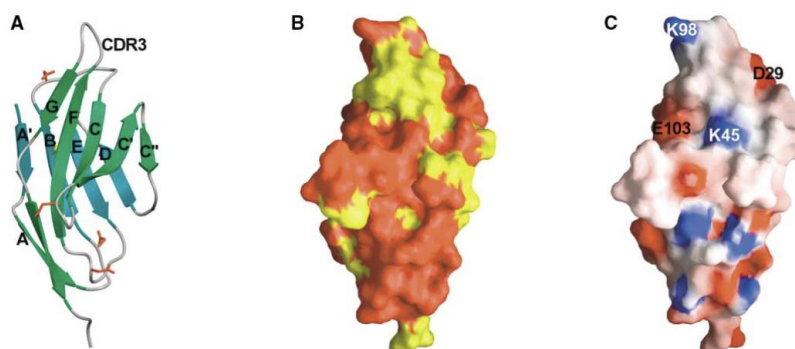


Figure 2. A) Ribbon diagram of the Ig v-type domain of murine PD-1. B) Hydrophobicity of the molecular surface of murine PD-1. C) Electrostatic molecular surface of murine PD-1[6].

When it comes to immune tolerance in the tumor microenvironment, it is regulated by the PD-1/PD-L1 signaling pathways. When PD-1/PD-L1 and PD-L2 are activated, they can activate T cells, promote cell proliferation and secretion of cytotoxicity, and contribute to the degradation of anti-tumor immune response in cancer patients. Persistently infected tumors and pathogens can thus evade T-cell-mediated tumor-specific and pathogen-specific immunity. Fig 2 shows the molecular structure of PD-1. PD-1 /PD-L1 is a type I transmembrane glycoprotein, which mainly includes transmembrane domain, variable (IgV) extracellular domain, and cytoplasmic tail as signal transduction or scaffold protein. The interfaces created by PD-1 and its different ligands are distinct, which accounts for PD-1's affinity for PD-L2. For instance, the conserved tryptophan W110 in PD-L2 establishes the most interactions with PD-1 residues but is replaced by alanine in PD-L1. When W110 in PD-L2 is deleted or mutated to alanine, the binding affinity for PD-1 is decreased. The PD-1 pathway to inhibit the activation of T-cells was reported as well. Similar to CTLA-4, PD-1 signaling initiates a series of downstream events that eventually attenuate T-cell activation and activity. As shown in Fig 3A, the activated PD-1 would inhibit T-cell receptor proximal kinases by the recruitment of SHP-2 which causes the attenuation of Lck-mediated phosphorylation of ZAP-70 and other unknown effects. Fig 3B shows the PI3K-Akt pathway, it is a vital signaling target of PD-1 mediated inhibition, it is different from the inhibition mechanism of CTLA-4; instead of directly suppressing activation of Akt, PD-1 can block PI3K. Fig 3C demonstrates another key pathway, the Ras-MEK-ERK pathway; By changing the activation of Ras-MEK-ERK, PD-1 is likely to have an impact on a wide range of downstream biochemical targets that are controlled by this path. It is important to note that PD-1 does not block all signals. It makes certain transcription factors, like BATF, more active, as shown in Fig 3D [7]. Nivolumab, for example, is a genetically modified monoclonal antibody that blocks PD-1 and PD-L1.

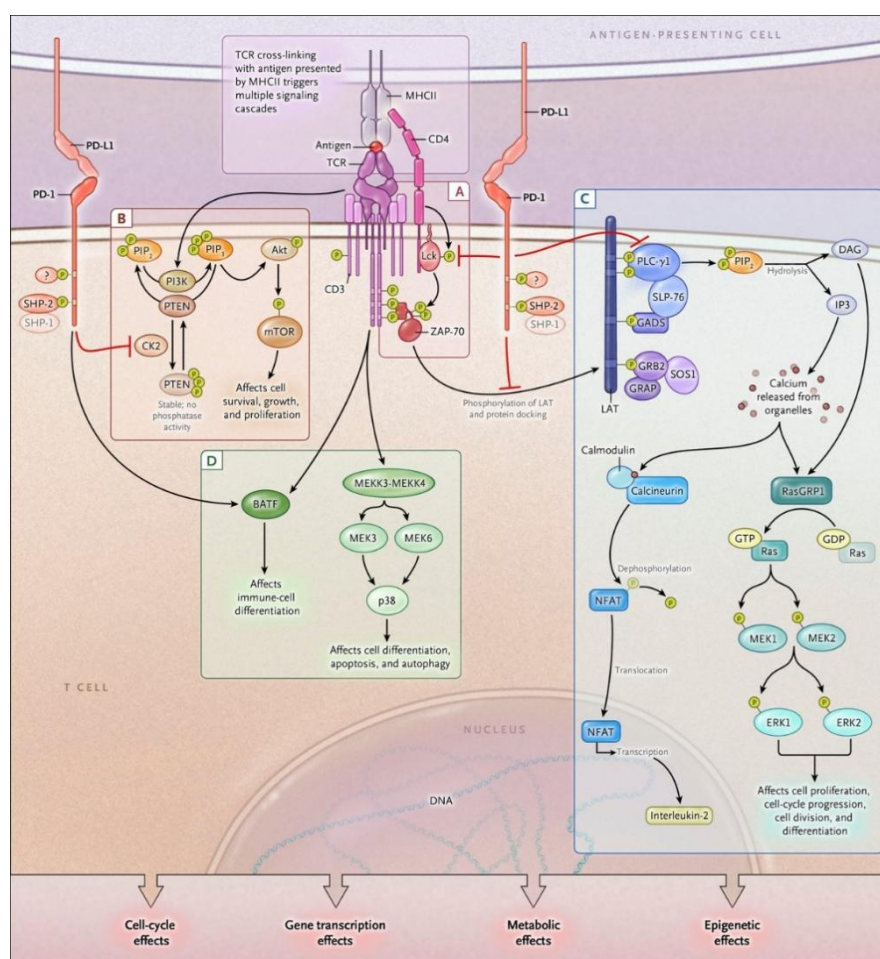


Figure 3. Effects of PD-1 on major signaling pathways in T cells [7].

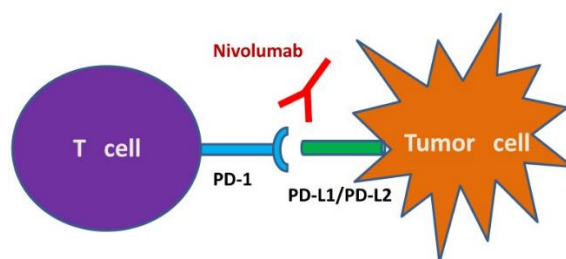


Figure 4. The mechanism of nivolumab [6].

3.2. Inhibitors that block PD-1/PD-L1

3.2.1 Nivolumab (BMS-936558)

Nivolumab is a immunoglobulin IgG4 antibody that targets PD-1 and is now being tested in advanced stage NSCLC clinical studies [8]. Nivolumab has a hinge region mutation (S228P) that affects the interaction between Fc and the serum IgG4 molecule negatively, thereby improving stability and reducing variability. It has a high affinity for PD-1, suppresses PD-L1 and PD-L2 interactions, and improves memory responses to the proliferation of tumor antigen-specific T cells [9]. A recent study found that when patients with high PD-L1 expression received PD-1 or PD-L1 inhibitors alone, they had a substantially smaller risk of mortality than patients in the control groups [10]. CheckMate-057, a phase III randomized study, compared the effectiveness of nivolumab with docetaxel in patients with non-squamous cell NSCLC. Patients receiving nivolumab showed improved one-year overall survival (OS) rate (50.5% vs. 39.0), progression-free survival (PFS) rate at 12 months, and OS at the primary endpoint (12.19 vs. 9.36 months) over those who received docetaxel [ClinicalTrials.gov identifier: NCT01673867]. Nevertheless, the amount of completed clinical trials evaluating the effectiveness of nivolumab in NSCLC has still been limited.

3.2.2 Pembrolizumab (MK-3475)

Pembrolizumab is a specific IgG4 monoclonal antibody that targets PD-1 and blocks inhibitory signals in T cells. In Keynote-010, which is an open-label, randomized phase II/III trial comparing the effectiveness of two doses of pembrolizumab (2mg/kg and 10mg/kg) and docetaxel in treating NSCLC patients, pembrolizumab has shown improved results, especially in participants who were strong PD-L1 positive. Patients who received 10mg/kg of pembrolizumab have demonstrated the best OS (17.3 vs. 14.9 vs. 8.2 months) over those who received 2mg/kg of pembrolizumab or docetaxel [ClinicalTrials.gov identifier: NCT01905657]. In KEYNOTE-024, patients showed superior PFS rate at month 6 (62.1% vs. 50.3%), OS rate at month 6, and ORR of pembrolizumab monotherapy over chemotherapy [ClinicalTrials.gov identifier: NCT02142738]. Hence pembrolizumab has demonstrated great efficacy in clinical trials for NSCLC.

3.2.3 Atezolizumab (MPDL-3280A)

Atezolizumab binds to PD-L1 and suppresses it from interacting with its ligase, PD-1. It's made to avoid antibody-dependent cell-mediated cytotoxicity (ADCC) in activated T cells with PD-L1 expression. POPLAR, a randomized, open-label phase II research, compared atezolizumab to docetaxel in NSCLC patients after platinum failure. Patients who received atezolizumab demonstrated better OS and a higher objective response rate (15.3% vs. 14.7%) than those who received docetaxel [ClinicalTrials.gov identifier: NCT01903993]. In IMpower131, a phase III randomized trial assessing the effectiveness of atezolizumab in the combined modality in stage IV squamous NSCLC, patients receiving atezolizumab as well as Carboplatin+Nab-Paclitaxel showed improved PFS in the intent-to-treat (ITT) population (6.5 vs. 5.6 months) and OS in the ITT population (14.3 vs. 13.2 months) over patients who received Nab-Paclitaxel + Carboplatin [ClinicalTrials.gov identifier: NCT02367794]. Therefore, atezolizumab is effective in managing NSCLC.

3.2.4 Durvalumab (MEDI4736)

Durvalumab is a IgG1 antagonistic antibody which can bind to PD-L1 with high affinities [9]. In PACIFIC trial, a phase III randomized clinical study, durvelumab was compared to placebo in treating patients that had not progressed after receiving definitive, concurrent, platinum-based chemoradiation therapy. Patients receiving durvelumab have shown improved ORR (30.0% vs. 17.8%) and PFS (16.8 vs. 5.6 months) over those who received placebo. The median and upper limit of 95% confidence interval (CI) of OS have not been reached yet [ClinicalTrials.gov identifier: NCT02125461]. However, since duvalumab was only approved by FDA in 2018, many clinical trials assessing the effectiveness of duvalumab in NSCLC are still in the process.

Table 1. Other information of checkpoint inhibitors [8].

Drug	Manufacturer	FDA approval	Indication
Nivolumab	Bristol-Myers Squibb (Princeton, New Jersey)	March 2015	For metastatic/unresectable/recurrent NSCLC
Pembrolizumab	Merck (Kenilworth, New Jersey)	October 2015	For stage III NSCLC which is metastatic/cannot be managed by surgical resection or definitive chemoradiation with PD-L1 expression
Atezolizumab	Genentech/Roche (San Francisco, California)	April 2016	For advanced stage NSCLC as second-line therapy
Durvelumab	AstraZeneca	February 2018	For unresectable stage II NSCLC which has not progressed after receiving concurrent platinum-based chemotherapy and radiation treatment

4. Combined therapies of PD-1/PD-L1

4.1. Combination with stereotactic body radiation therapy

Despite the promise of PD-1/PD-L1 inhibitors, there still remain numerous patients with advanced NSCLC that can not respond to these drugs. Radiation has been proven to have powerful immune system regulatory effects in preclinical and clinical trials, potentially creating a supportive antitumor immunological microenvironment [11].

Stereotactic body radiation therapy (SBRT) can give high dosages to relatively small target lesions, which means powerful immunologic adjuvants, and therefore controlling over 80% of the local tumor and significantly improving prognosis with a lower toxicity risk [12]. Thus, compared with conventional radiotherapy, the superiority of SBRT makes it a better option for combined therapies of PD-1/PD-L1 inhibitors to attain improved survival.

SBRT has a positive immune regulatory effect on the innate and acquired immune systems. Hypofractionated SBRT can promote the expression of ICAM-1, MHC-1, and Fas, which may be downregulated in the immune system and contribute to resistance and evasion. Higher expression level of these markers speeds up the immune system's response to cell abnormalities, resulting in improved antitumor effects [13]. Moreover, it has been found that hypofractionated SBRT can help release an adequate number of tumor-associated antigens (TAAs), which will further be used by DCs and regulate immune activation signals transmission [14]. In addition, hypofractionated SBRT can recruit immune cells to irradiated tissues by promoting the release of cytokines and changing the vascular phenotype. Studies have reported that higher doses of radiation can cause intracellular stress, resulting in the occurrence of immunologic cell death (ICD), which may be able to promote the expression of DCs and the cross-priming of cytotoxic T lymphocytes (CTLs). It has also been found that the amount of CTLs increased and the amount of Tregs decreased in mouse tumors after receiving a high dose of radiation [15]. However, SBTR may also lead to an immunosuppressive microenvironment. It has been found that radiation might result in an increase in transforming growth

factor β (TGF β), which induces the CD4⁺ T cells to become Tregs, suppressing the antitumor immune response. Furthermore, radiation may also unexpectedly induce higher expression of PD-L1, which induces increased immunosuppression via interacting with the PD-1 receptor, leading to stronger resistance to radiation [16].

When combining SBTR with PD-1/PD-L1 inhibitors, the trigger of immunizing cells and the upregulation of PD-L1 caused by SBTR provides a microenvironment that support PD1/PD-L1 inhibitors to work. Moreover, the functions of PD-1/PD-L1 inhibitors in reducing immunosuppression can also attenuate radio-resistance, thus leading to better survival.

The efficacy of SBRT followed by pembrolizumab was used in a phase I research for metastatic NSCLC, with a median OS (mOS) and median PFS (mPFS) of 9.6 months and 3.1 months [17]. In another multi-center phase II trial, patients receiving pembrolizumab after SBRT had superior mPFS and mOS over patients who received pembrolizumab as monotherapy [18].

4.2. Combination with chemotherapy

Despite the significant development of new cancer treatment, chemotherapy still remains the main method for treating cancer. However, conventional chemotherapy for cancer is usually immunosuppressive and has been connected with resistance to drugs and regeneration of malignancies [19]. Chemotherapy can directly or indirectly modify immune responses, whereas ICIs reactivate immunological responses. As a result, chemotherapeutic medicines and CPIs may have complementary processes.

The effectiveness and safety of chemotherapy combined with the therapeutic vaccine TG4010 and nivolumab for advanced stage non-squamous NSCLC patients have been assessed in a study, patients showed an ORR of 32.5% and a disease control rate (DCR) of 75.0%. The OS was 14.9 months with the upper limit still expending [ClinicalTrials.gov identifier: NCT03353675]. In CheckMate-012, a phase I randomized clinical trial of nivolumab in combined modality in patients with stage IIIB/IV NSCLC, the group receiving Nivolumab + Pemetrexed + Cisplatin achieved an ORR of 46.7% and a PFS rate at week 24 of 68.4%. However, the rate of patients who had serious adverse events (SAE) or adverse events (AE) in this group is also relatively higher, which is 66.7% [ClinicalTrials.gov identifier: NCT01454102]. In KEYNOTE-021, a randomized, open-label trial to determine the effectiveness of pembrolizumab in conjunction with chemotherapy among patients with unresectable or metastatic NSCLC, the group receiving pembrolizumab plus carboplatin plus pemetrexed had a superior PFS (13.0 vs. 8.9 months) over the group receiving placebo plus carboplatin plus pemetrexed with the median OS still expending [ClinicalTrials.gov identifier: NCT02039674].

4.3. Combination with other immune checkpoint inhibitors

Because immunological checkpoints on T cells are triggered in different ways, for instance, CTLA-4 is stimulated in lymphoid tissue while PD-1 and PD-L1 are activated in the tumor microenvironment, it is reasonable to believe that combining different CPIs may improve clinical results. In CheckMate-012, a randomized phase I study assessing the efficacy of nivolumab in combined modality in stage IIIB/IV NSCLC patients, those receiving Nivo 0.003 g/kg Q2W + Ipi 0.001 g/kg Q12W had the highest PFS rate at week 24 of 72.4% among all treatment groups. However, the rate of participants who experienced SAE or AE in this group is 61.4%, which is also relatively higher [ClinicalTrials.gov identifier: NCT01454102].

5. Limitations and potential improvements of PD-1/PD-L1 treatments

5.1. The immune-related adverse events

Nivolumab medication, like other cancer medicines, is frequently accompanied by side effects such as tiredness, nausea, vomiting, cough, dyspnea, constipation, reduced appetite, diarrhea, pyrexia, , dermatitis, and headache [20]. These side effects are possible limitations of PD-1 inhibitors for certain individuals; nevertheless, adverse events occur less and are not as severe as with

ipilimumab, and the majority of patients who quit owing to toxicity still exhibit a long-lasting and continuing response [21]. Larger trials are able to indicate how to appropriately control these side events while administering anti-PD-1 mAb immunotherapy without compromising clinical efficacy. Initially, immune-related adverse events were organ-specific, rather than impacting many organs at the same time. Following that, the beginning of toxicities is usually postponed. While utilizing ipilimumab and nivolumab, they would be deferred until the fourth and tenth weeks, respectively [22]. Although these adverse effects are typically controllable, which means they may be minimized by dosage change, steroid administration, and treatment withdrawal [23], they can also be deadly in rare situations and are unavoidable due to dose decreases, sometimes causing lifelong harm to patients.

5.2. The innate and acquired resistance

Patients that do not react to PD-1/PD-L1 blocking have ‘innate resistance’, whereas those who respond briefly before their cancers develop have ‘acquired resistance’ [24]. It is necessary to find an accurate method of anticipating which individuals are likely to benefit from anti-PD-1/PD-L1 therapies. At the moment, neither intrinsic nor learned resistance to ICI treatment is entirely understood.

The failure of the localization of host CD8⁺ T cells to a tumor can be explained as a lack of suitably immunogenic tumor antigens for T-cell identification [25]. This might be important to consider in cancers that are either not considerably dedifferentiated from their origin tissue or have an inadequate mutational load to release tumor antigens capable of eliciting a concentrated CD8⁺ T cell response. As a result of the lack of T lymphocytes that can detect distinct tumor antigens, the tumors are resistant to PD-1/PD-L1 blocking treatment. One strategy to solve this issue is to provide the individuals with T cells that target antigens that may not be naturally immunogenic.

Despite efficient CD8 T cell identification, tumors may have intrinsic primary resistance to PD-1/PD-L1 inhibition. This can emerge as a result of a tumor's lack of interferon-gamma (IFN) responsiveness, which is able to occur in primary as well as acquired resistance to checkpoint inhibition treatment. IFN is generated by CD8⁺ T cells that have detected and attracted a specific tumor antigen. It is the main mechanism of enhancing MHC expression and antigen presentation, attracting new T cells to tumors, and exerting straightforward anti-proliferative effects and death in cancer cells [26]. PD-L1 expression can be continuously expressed in some situations, in addition to indicating dynamic IFN response. Although the prognosis consequences of constitutive PD-L1 expression are not always evident, several are linked with a weak response to PD-1/PD-L1 checkpoint suppression. Individuals with EGFR mutations and ALK rearrangements had low reaction rates to PD-1/PD-L1 inhibition in NSCLC. Individuals with these mutations have high PD-L1 expression rates even though there is a little or nonexistent CD8⁺ T cell infiltration, showing that the PD-L1 expression was constitutive, instead of driven by local inflammatory stimulation. Furthermore, preclinical models of NSCLC with EGFR mutations and ALK rearrangements showed that the genetic changes play a key role in constitutive PDL1 expression observed in these tissues [27]. Though the pathway behind the apparent immune exclusion produced by these mutations is unknown, they highlight the risk of forecasting a tumor's responsiveness to PD-1/PD-L1 blocking treatment based only on the occurrence of a single marker.

6. Conclusion

PD-1 and PD-L1 inhibitors have been widely used in the management of NSCLC. They have demonstrated great effectiveness in clinical trials both alone and in combined modality. The application of combination therapy opens up broad prospects for the treatment of NSCLC and maybe solutions for tumor cell immunosuppression and immune escape. Nevertheless, adverse events resulting from PD-1 and PD-L1 inhibitors still remain a concern. In the future, further study is urgently needed to gain a better understanding of the mechanisms of immune-related adverse events so as to improve treatment.

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