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PD-1 Immune Checkpoint Inhibitor Therapy Malignant Tumor Based on Monotherapy and Combined Treatment Research

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Abstract

Recently, immunotherapy has become the fourth pillar of cancer treatment in addition to surgery therapy, chemotherapy, and radiation therapy. The inhibitors of programed cell death protein 1 (PD-1) and its ligand PD-L1 are the new stars in immunotherapy, as they can overcome tumor immunosuppression. However, the efficacy of PD-1 inhibitors still needs to be further developed for clinical treatment. Therefore, research into treatment with anti-PD-1 drugs has emerged as a new development field. This review provides novel insights into the role and mechanism of PD-1 combination anti-tumor therapy, thereby promoting its clinical application in anti-tumor immunotherapy.

Keywords

PD-1, PD-L1, anti-tumor, mechanism, immunotherapy

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Introduction

Programed cell death protein 1 (PD-1) is an important co-inhibitory signal on the T cells and plays a negative regulatory role in the immune response.[1] PD-1 bound to PD-L1, PD-L2, or the corresponding antibodies can play an immunosuppressive role around the tumor, resulting in the down-regulation of T cells activity and promoting tumor cells evasion of immune surveillance.[2,3] Unlike traditional treatments that directly kill large areas of tumor tissue, anti-PD-1 immunotherapy blocks this signaling pathway and activates an immune response that inhibits tumor cell growth.[4] According to anti-PD-1 clinical treatment data, this treatment is more effective for cancer patients whose level of PD-L1/PD-L2 expression is more than 50% on the tumor cell surface.[5,6] To improve the clinical efficacy of anti-PD-1, researchers have turned their attention to the combination therapy.[7,8] This article reviews the anti-tumor clinical effect and signaling mechanism of PD-1 and provides a theoretical reference for PD-1 combination as a clinical immunotherapy for anti-tumor.

PD-1 Is Expressed in Tumor Cells

PD-1 is mainly expressed on immune cells such as T cells, B cells, monocytes, and natural killer (NK) cells.[9] The mature form of the PD-1 protein contains 268 amino acids (aa) including the cytoplasmic domain (94 aa), hydrophobic transmembrane domain (27 aa), and extracellular domain (147 aa). The PD-1 protein has an immunoreceptor tyrosine-based switch motif (ITSM) and immunoreceptor tyrosine-based inhibitory

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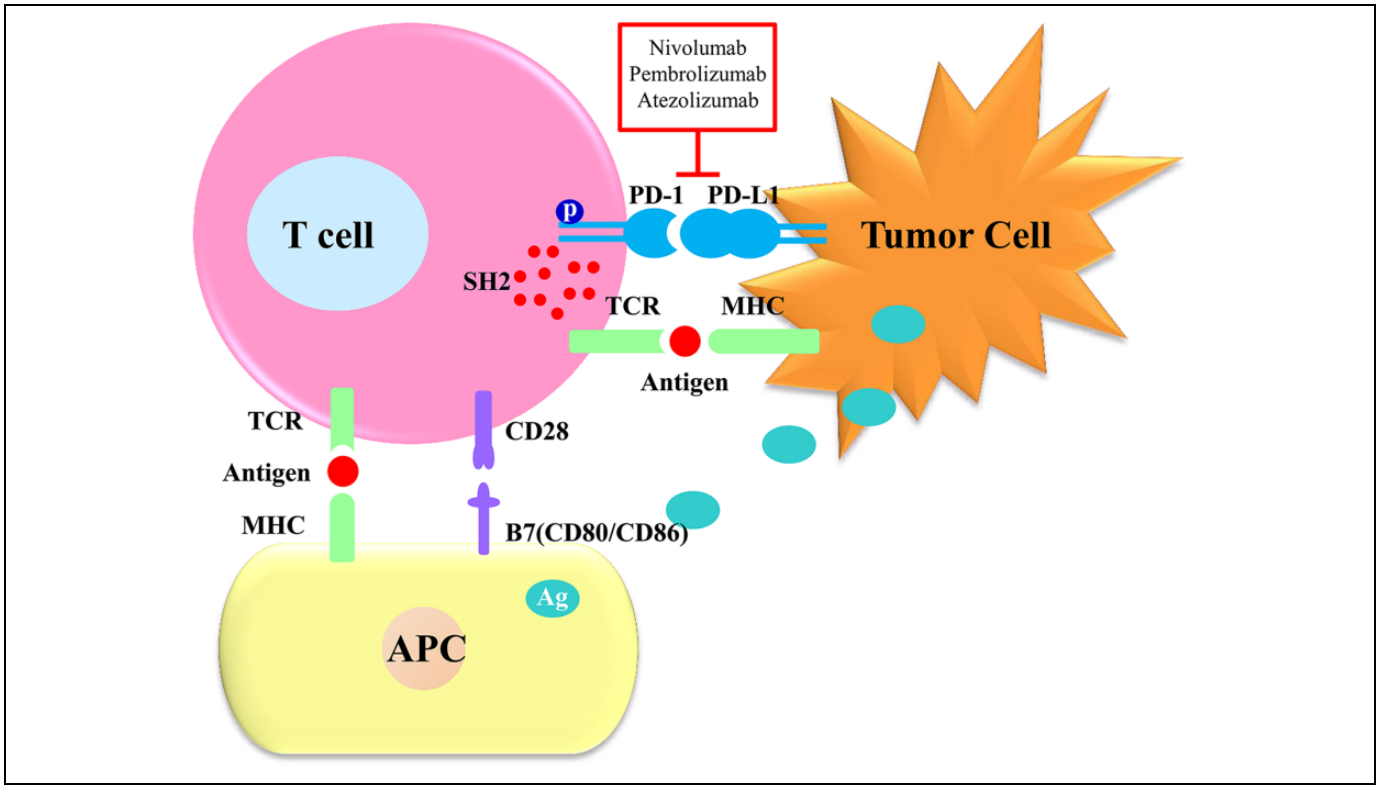

Figure 1. PD-1/PD-L1 signaling pathway. When PD-1 binds to PD-L1 that is expressed on the surface of tumor cells, it induced PD-1 phosphorylation of the intracellular N-terminal ITIM, thereby recruiting SH2 domain-containing protein tryrosine phosphatase-2 (SHP-2) to C-terminal ITSM tyrosine. The TCR of T cells binds to the MHC presented by APC to complete antigen recognition and can secrete antibodies to tumor cells. The above procedure can be reversed by PD-1 inhibitors (Nivolumab, Pembrolizumab and Atezolizumab). In addition, CD28 and ligands (CD80 and CD86) are exposed to the tumor microenvironment, T cells become unreactive or eliminated by programed cell death.

motif (ITIM) at the end of the cytoplasmic segment tail, which are important structural foundations for PD-1 to exert its immunosuppressive functions. The specific structure of PD-1 corresponds to its unique function.[10]

In normal cases, T-cell activation induces PD-1, initiating a feedback suppression mechanism to prevent excessive activation of the T-cell receptor (TCR).[11] In cancer patients, PD-1 binds to PD-L1/PD-L2 located on the signaling structure of tumor cells, inducing N-terminal ITIM phosphorylation in the cytoplasm of PD-1 and recruiting activated protein tyrosine phosphatase (SHP-2) turn to the C-terminal ITSM (Figure 1).[12] These activities lead to inhibition of the phosphatidylinositol-3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway and enhancement of caspase activity, thereby activating PD-1-mediated immune cell cycle arrest, reducing cytokine production, and down-regulating immune cell metabolism and many other immunosuppressive responses.[13,14] PD-1 interaction with PD-L1 can inhibit T cell proliferation and differentiation, resulting in decreased T cell function and even apoptosis, ultimately allowing tumor cells to escape the immune system.[15] Therefore, PD-1/PD-L1 blockade therapies have garnered much attention, as they have proven efficacious for the treatment of many cancers. In a previous study, the PD-1 gene was knocked out in cytotoxic lymphocytes (CTLs) to evaluate its effects on the anti-tumor activity of CTLs against multiple myeloma (MM). After knockdown, the secretion of cytokine tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) was significantly increased, enhancing the cytotoxic effects of CTLs on tumor cells and ultimately inducing the apoptosis of tumor cells, further confirming the important role of anti-PD-1 in tumor suppression.[16] Anti-PD-1 (nivolumab and pembrolizumab) or anti-PD-L1 drugs can significantly inhibit the invasion and migration ability of tumor cells, thereby enhancing TCR signal transduction and the function of T cells, which ultimately inhibits the growth of tumor cells. In clinical research, anti-PD-1 drugs are mainly used in solid tumors (e.g., lung cancer, metastatic melanoma), and their efficacy against other cancers is being studied in large-scale clinical trials in patients with cancers such as renal cell carcinoma, bladder cancer, and Hodgkin lymphoma.[17,18]

Anti-PD-1 Prevent Malignant Tumors

Tumor immunotherapy is a current research hotspot. The successful development and clinical application of PD-1/PD-L1 inhibitors have elevated tumor immunotherapy to a new level.[19] In 1992, the cDNA of PD-1 was discovered by the Japanese scientist Honjo; however, the structure and function of PD-1 were unknown. In 1994, cDNA encoding mouse PD-1

was isolated from apoptosis-cells by subtractive hybridization. The human T cell cDNA library was screened by mouse PD-1 probe, and the cDNA encoding human PD-1 protein was isolated, and then the human PD-1 gene was also mapped to 2q37.3.[20,21] During the activation and differentiation of T lymphocytes, the B7 molecules interacting with PD-1 were reported in the next 2-5 years. Currently, anti-PD-1 (nivolumab, pembrolizumab) drugs have been used for the treatment of various cancers. In addition, new PD-1 such as Spartalizumab, Cemiplimab and MEDI0680 are also continuously entering clinical trials.

A related mechanism analysis has shown that anti-PD-1 activates dendritic cells (DCs) to present tumor-associated antigens to T cells, simultaneously blocking the release of NF-κB-dependent cytokines and significantly mitigating tumor growth and prolonging the life of mice. Research has also shown that anti-PD-1 can enhance the killing effect of CIK cells on A520 lung cancer cell line.[22,23] Anti-PD-1 significantly upregulates NK cell ($CD3^-CD56^+$) activity by cluster of differentiation 16 (CD16) and interleukin 2 (IL-2) and increases the migration of NK cells to MM target cells.[24,25] In the tumor microenvironment, anti-PD-1 promotes the T-cell anti-tumor immune response during the effector phase. Anti-PD-1 reverses the function of $CD8^+$ T cells, promoting intracellular IFN-y accumulation and reducing IL-10 secretion. Accumulating research suggests that T cells expressing the PD-1 antigen chimeric receptor (ACR) in the signaling domain increases PI3K/AKT activity, increases cytokine secretion, and up-regulates B-cell lymphoma extra-large, which not only promotes T-cell activation but also prolongs the survival time of the T cells. A previous study also found that PD1-ACR can target U87 invasive tumor areas, significantly inhibiting the growth of metastatic tumors, thereby prolonging the survival of U87-bearing mice.[26]

Nivolumab Prevents Tumor Growth and Metastasis

Nivolumab is an IgG4 monoclonal antibody and the first approved PD-1 inhibitor. It competes with PD-L1 ligand on the tumor surface for PD-1 on T cells, significantly suppressing the PD-1 pathway to produce immunological checkpoint inhibitors.[27] Previous studies have shown that nivolumab has a sustained tumor response rate of 20%-30% for a variety of cancers. Due to the effective results of nivolumab in Phase I trials, phase III evaluation was conducted in 3 different cancers (melanoma, renal cell carcinoma, and non-small cell lung cancer), an extraordinary moment in the development of an anticancer drug.[28] Topalian pointed out that this new drug has broken through the 10%-15% limit of sustained tumor response, which has hampered the development of cancer immunotherapy for the past 30 years. In a meta-analysis of 3404 patients from 20 studies, it was shown that nivolumab may cause a sustained response, with a median post-progression survival of approximately 1 year in patients with advanced non-small cell lung cancer (NSCLC).[29] Nivolumab is widely used for the clinical treatment of NSCLC. Yet, its therapeutic effect is unclear for lung cancer caused by interstitial lung disease (ILD). Kanai counted the medical records of 216 patients with NSCLC who received nivolumab and found 26 with ILD. The effects of nivolumab were measured by response rate (RR), duration of progression-free survival (PFS), and lung toxicity (incidence, severity, and prognosis) in the ILD and non-ILD groups. The results showed that the overall incidence of NSCLC in the ILD group was higher than that in the non-ILD group after nivolumab treatment. Furthermore, more than 50% of patients with NSCLC in both groups had symptoms that improved over time.[30]

Pembrolizumab Prevents Tumor Growth and Metastasis

Pembrolizumab is a humanized anti-PD-1 antibody that has been extensively studied in a variety of malignancies. It recognizes PD-1 by intermolecular direct and water-mediated hydrogen bonds (wHBs), non-conventional hydrogen bonds (nHBs), hydrophobic contacts, and salt bridges (SBs).[31] Pembrolizumab has a similar anti-tumor mode of action of nivolumab. In 2016, pembrolizumab was approved by the U.S Food and Drug Administration for untreated patients with metastatic NSCLC or those treated with platinum-based chemotherapy. Pembrolizumab induces an overall response rate (ORR) of 21%-34% in melanoma, and the root cause of the low survival rate is the difficulty of target therapy. In stage III/IV unresectable melanoma, pembrolizumab is superior to ipilimumab. Likewise, pemizumab induces an ORR of 19%-25% in NSCLC. Based on the above results, pembrolizumab has been approved for the treatment of advanced melanoma and NSCLC. Preliminary data have shown that the ORR of pembrolizumab is approximately 20%-50% in malignant tumors including lymphoma and other solid tumors.[32] Pembrolizumab has been found to have durable responses in patients with advanced NSCLC through years of clinical research. These findings have changed the current therapeutic mode in advanced NSCLC, adding a new treatment option for patients.[33] In recent years, pembrolizumab has been approved for use in a growing number of cancer types. Frank examined the efficacy of pembrolizumab in the treatment of advanced melanoma and its associated clinical outcomes for more than 4 years in the United States. Pembrolizumab was given to 315 (59%), 152 (29%), and 65 (12%) patients. Overall, the 1- and 2-year survival rates of pembrolizumab were 61% and 48%, respectively; lactate dehydrogenase levels returned to normal (relative to elevation), and overall survival (OS) was significantly improved. These findings demonstrate the effectiveness of pembrolizumab in the actual clinical setting.[34] Another study found that pembrolizumab was used for intravenous infusion (escalating doses 2 or 10 mg/kg) until disease progression or severe toxicity. During the study, 80% of patients had nausea and fever, and it elevated aspartate aminotransferase/alanine aminotransferase. Collectively, the therapeutic safety profile of pembrolizumab in Japanese patients is similar to those previously reported for Caucasian patients.[35]

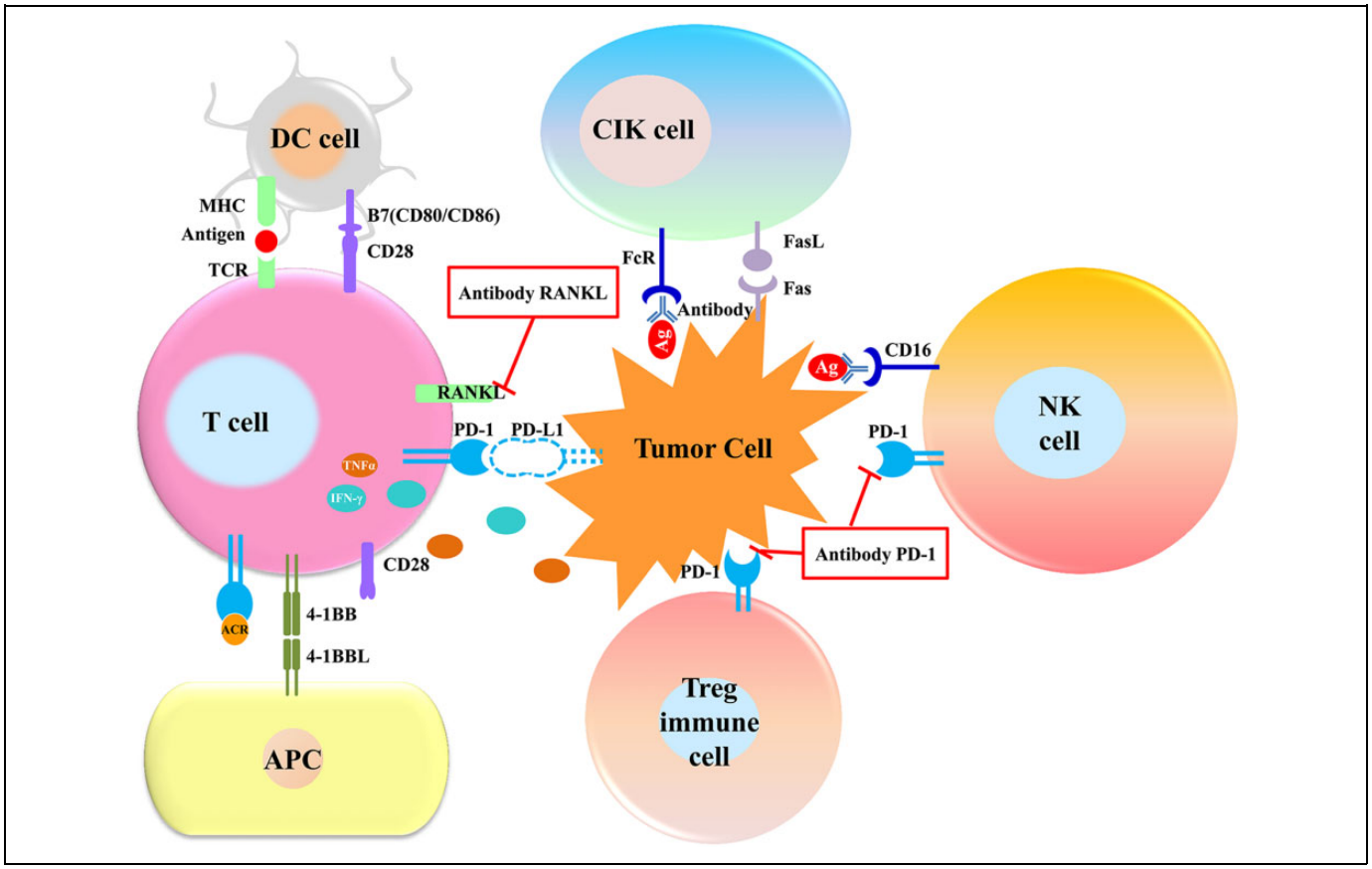

Figure 2. Anti-PD-1 combined with other cells inhibiting tumor growth and metastasis. The addition of anti-PD1 to TILs increased RANKL expression, and anti-RANKL directly reduced PD-L1 expression on the non-lymphoid component tumor microenvironment. Therefore, the addition of RANKL blocker can improve the efficacy of PD1, CTLA4 and RANKL inhibitors, and increase the proportion of IFNγ and TNFα in tumor-infiltrating $CD4^+$ and $CD8^+$ T cells. In addition, CD28 on T cells binds to ligands on DC cells to regulate tumor cells. T cells were activation of co-stimulatory molecules by 4-1BB and 4-1BBL enhances the survival of primary T cell responses and memory T cells. Besides, anti-PD-1 significantly inhibits tumor growth by activating DCs to present tumor-associated antigens to primary T cells. When CIK is activated by binding of the antigen-antibody complex to the FcR, toxic particles are excreted to the cells, and binding of FasL and Fas, the tumor cells undergo apoptosis. In addition to the release of toxic particles and Fas/FasL, CD16 expressed on the surface of NK cells binds to antibodies and kills tumor cells.

Analysis of Anti-PD-1 Combination Therapy to Inhibit Tumor Growth and Metastasis

Anti-PD-1 has anti-tumor effects, but its effective anti-cancer rate is not ideal. How to increasing the survival of patients is still a hot issue in current research. A great amount of data have shown PD-1 and B and T lymphocyte attenuator (CD272) simultaneously inhibit the signal at the interface of major effector T cells and T-cell antigen presenting cells, which provides a theoretical basis for the combination of blocking antibodies in cancer immunotherapy.[36,37] Researchers have found through numerous studies that anti-PD-1 combination with CTLA4, lymphocyte activating gene 3 and T cell immunoglobulin and mucin domain-containing protein 3 antibodies significantly increase IFN-γ and TNFα.[38,39] Besides, combined treatment restores activation and proliferation of $CD4^+$ and $CD8^+$ T cells, significantly enhancing the function of effector T cells and enhancing the tumor's immune inhibition response. At the same time, combined treatment prevents autoimmune diseases by reducing regulatory T cell activity and upregulating IFN regulatory factor. In summary, anti-PD-1 combined treatment can inhibit the growth and metastasis of mouse ovarian cancer cells, H22 liver cancer cells, and MM cells, significantly improving the OS rate of mice, and has an effective therapeutic effect.[40-42] In addition, (S)-(-)-n-[2-(3-hydroxy-1H-indol-3-yl)-methyl]-acetamide (SNA), a specific inhibitor of PI3Kδ, play an inhibitory on myeloid-derived suppressor cells (MDSCs), then trigger changes in the tumor microenvironment and enhance the activation and infiltration of T-cells. The combination of SNA with anti-PD1 can activate $CD8^+$ T cell-selective infiltration by PI3Kδ/γ, having good therapeutic effects.[43] Recently, irreversible electroporation combined with anti-PD-1 was found to activate $CD8^+$ T cells by DCs to improve the tumor microenvironment. It also promotes micro-vascular production that in turn promotes the infiltration of T cells of tumor-associated antigens, significantly inhibiting tumor growth, but has no effect on normal tissues, prolonging the lifespan of orthotopic pancreatic tumor mouse models (Figure 2).[44,45] The effects of the 2 PD-1

inhibitors will be described in detail. Reem et al[46] found that the co-blockade of PD-1 and PD-L1 further up-regulates the co-expression of TIM-3 and LAG-3 on $CD4^+CD25^+$ T cells and $CD4^+CD25^+FoxP^{3+}Helios^+$Tregs in the presence of TNBC cells, but not in non-TNBC cells. The results indicate that the emergence of compensatory inhibition mechanisms is most likely mediated by Tregs and activated non-Tregs. Our results indicate the emergence of compensatory inhibitory mechanisms, most likely mediated by Tregs and activated non-Tregs, which could lead to the development of TNBC resistance against PD-1/PD-L1 blockade.

At present, the sensitivity of chimeric antigen receptor-T (CAR-T) cell therapy is high, but damage caused by off-target effects can lead to serious side effects. Recent clinical results have shown that CAR-T therapy combined with anti-PD-1 is frequently used for solid tumor treatment.[47] However, it remains unknown whether it can truly treat cancer; more clinical data are needed for confirmation. Nevertheless, combination therapy is showing promise in treating cancer and producing durable responses.

Nivolumab Combined Cohort Analysis to Inhibit Tumor Growth and Metastasis

Data over the past several decades have shown that nivolumab-based immunotherapies have clinical efficacy, leading to approval of the combination of nivolumab and ipilimumab in treating patients. One study showed 64 patients treated with nivolumab, among which 26 patients discontinued the drug due to disease progression or AEs. The 26 patients were treated with nivolumab in combination with platinum doublets, monotherapy, with a disease response rate of 34.6% (9 patients) and 57.7% (15 patients) and disease control rate of 73.1% (19 patients) and 19.2% (5 patients). A multivariate regression analysis showed that nivolumab combined with platinum doublets is more effective than monotherapy.[48] In a trial of patients with late-phase melanoma, we randomly assigned patients who were not previously treated in a 1:1:1 ratio: ①Nivolumab+Ipilimumab group; ②Nivolumab+placebo group; ③Ipilimumab+placebo group. In subsequent follow-up visits, the overall 3-year survival rate of nivolumab combination with ipilimumab was 58%, in the nivolumab group was 52%, and in the ipilimumab group was 34%. In summary, the OS of nivolumab and ipilimumab was significantly longer compared to ipilimumab or nivolumab alone.[49]

Pembrolizumab Combined Cohort Analysis to Inhibit Tumor Growth and Metastasis

With the widespread use of anti-PD-1 therapy in melanoma patients, problems have followed, of which brain metastasis is becoming more common.[50] From January 2014 to December 2015, Erik evaluated the safety of 21 patients receiving pembrolizumab combined with stereotactic radiosurgery (11 cases), hyperfractionated radiation (7 cases), and whole brain therapy (3 cases). In the initial response, all treatments were well tolerated, and no grade 4 or 5 toxicity was observed. For brain metastases treated with combination therapy, 70% (16/23) showed complete remission (CR, $n = 8$) or partial remission (PR, $n = 8$) at the first scheduled follow-up (median 57 days post-treatment). The survey demonstrated that pembrolizumab combination with radiation therapy was safe for patients with metastatic melanoma, especially at the first visit, effectively reducing the size of brain metastases. In general, these results are superior to cases of individual treatment.[51] In a previous study, EDP1503 was able to activate a variety of systemic immune pathways; therefore, it was hypothesized that EDP1503 could be combined with immunological checkpoint inhibitors to achieve anti-cancer goals. Recently, the monoclonal microbial candidate for drug EDP1503 combined with pembrolizumab has been officially enrolled in the I/II clinical trial, and tumor patients who have recurrent rectal cancer and triple-negative breast cancer have been formally enrolled. This clinical trial will assess the safety, tolerability, and overall response rate of the immune response in 120 patients after combination therapy. Besides, initial clinical data are expected to be tested in mid-2020.[52] Dr. Humphrey Gardner affirmed the study and said that this clinical trial will explore the potential synergy between EDP1503 and pembrolizumab and provide the potential to treat multiple cancer types.

More PD-1 Inhibitors Cohort Analysis to Prevent Tumor Growth and Metastasis

PD-1 inhibitors are still under continuous development in anti-tumor. In addition to Nivolumab and pembrolizumab, more PD-1 inhibitors are gradually being discovered. Two other PD-1 inhibitors are introduced below. Cemiplimab (Libtayo) is the third PD-1 monoclonal antibody approved for marketing in the United States, and the first and only drug specifically approved for skin squamous cell carcinoma (CSCC). At present, clinical studies of cemiplimab cover a variety of solid tumors, including non-small cell lung cancer (NSCLC), glioblastoma, prostate cancer, ovarian cancer, cervical cancer, and thyroid cancer. The results of the phase III of the PD-1 monoclonal antibody cemiplimab (Libtayo), the first-line treatment of NSCLC, were announced at the ESMO meeting in 2020.[53,54] It is strong evidence that PD-1 monoclonal antibody has taken the first-line treatment of NSCLC. In the PD-L1 $\geq$50% intention-to-treat (ITT) population, the median follow-up time is about 10 months. The median overall survival (OS) data of cemiplimab reached (17.9 months-not estimable), and the median OS data of the chemotherapy group was 14.2 months (11.2-17.5 months). The median progression-free survival (PFS) of the cemiplimab group was 8.2 months (6.1-8.8 months), and the chemotherapy group was 5.7 months (4.5-6.2 months). The objective response rate (ORR) of cemiplimab group was 39.2%, and the chemotherapy group was 20.4%. The median remission of the cemiplimab group was 16.7 months (12.5-22.8 months), and the chemotherapy group was 6.0 months (4.3-6.5 months). Cemiplimab reduces the risk of disease progression or death by 43%. The above data show that cemiplimab has

obvious advantages in the first-line treatment of NSCLC patients with PD-L1≥50%. CT-011, a new type of anti-PD-1 antibody, seems to be specific cytotoxicity to NK cells trafficking, immune complex formation, and cytotoxicity for PD-L1$^+$ multiple myeloma (MM) tumor cells rather than normal cells. Jacalyn et al[55] show that lenalidomide down-regulates PD-L1 on primary MM cells and may enhance NK cell function through CT-011. CT-011 combined with lenalidomide should be considered for phase II clinical trials of MM patients. Studies have shown that 17 patients have received increasing doses of CT-011 (0.2-6 mg/kg). Blood samples were taken before treatment, immediately after treatment, 24 hours, 48 hours, 7th day, 14th day and 21st day. Within 21 days after CT-011 treatment, the percentage of CD4$^+$ in peripheral blood was observed to continue to increase. Pharmacokinetic analysis showed that the serum Cmax and AUC of CT-011 increased proportionally with the increase of dose. The results showed that the CT-011 is safe and well tolerated in this patient population and 33% clinical benefit was observed in patients.[56]

Anti-PD-Ls Prevent Malignant Tumors

A prospective study observed that PD-Ls (PD-L1/PD-L2) can be overexpressed in tumor cells such as ovarian cancer, meningioma, and melanoma, as well as can directly promote tumor growth.[57] Another study showed that the overexpression of PD-Ls in tumor cells is closely related to the recurrence and metastasis of tumor cells, and the expression level in cancer tissues is significantly higher than that in normal tissues.[58] In addition, it has been reported that there is no significant correlation between PD-L1 and PD-1 expression in cancer and lymph node tissues. However, IL-2 and IL-10 are involved in the regulation of PD-L1 expression and induction of tumorigenesis with lymph node metastasis, which may be one of the factors affecting prognosis.[59] Therefore, it suggests that common anti-PD-L1 can improve the survival rate and remission rate in cancer patients, which has a positive impact. Until now, anti-PD-L1 (atezolizumab, durvalumab, and avelumab) has been approved for the treatment of urothelial carcinoma, and several other drugs are still in early clinical trials stage. The discovery of anti-PD-L1 provides a new solution for cancer therapy. In the same period, it was shown that blocking PD-L1 can enhance the expansion of TILs and its function, and reverse the tumor immune CD8$^+$ T cells in the tumor microenvironment.[60,61] Studies have also proposed the role of immune cell infiltration and immune cell function in predicting the efficacy of PD-1/PD-L1 blockade therapy. Based on these mechanisms, researchers have proposed combined treatment strategies, and the importance of patient-specific treatment plans to prolong the life of patients.[62] To summarize, anti-PD-L1 can block the binding of PD-1 and PD-L1, up-regulate the growth and proliferation of T cells, enhance the recognition of tumor cells by T cells, which activate its attack and killing functions, and mobilize the body's own immune function to achieve resistance tumor effect.

Discussion

At present, immunological checkpoint inhibitors (ICPis) are mainly used in the treatment of anti-tumor immune response. After many years of research, PD-1/PD-Ls are an important immune-regulator factor. PD-1/PD-Ls inhibitors show target tumors with effects in blocking tumor growth and proliferation. Moreover, as the tumor evolves, the immune system of the body can be improved. Due to individual reasons and different types of cancer, 15%-40% of cancer patients show clinical responses. Other patients cannot benefit from anti-PD1/PD-L1 treatment. Some patients with clinical response will develop acquired resistance after the initial response. Therefore, understanding the mechanism of drug resistance is a necessary condition for improving the efficacy of anti-PD1/PD-L1. At present, researchers have identified the main drug resistance mechanisms for tumor, including insufficient tumor immunogenicity caused by loss of tumor antigens, cell and molecular suppression signals in the tumor microenvironment (TME), MHC dysfunction, and irreversible T cell failure, the immunosuppressive microenvironment, all of which may impair the durability of treatment. In this regard, researchers described a potential strategy for the combination of targeted Treg and ICPis to overcome this resistance and maximize the efficacy of treatment for cancer patients.[63-65]

Blocking PD-1/PD-L1 signal or co-processing other cooperative stimulation signals has shown amazing effects in inhibiting tumor growth. Based on the search of PD-1/PD-L1 inhibitors, the combination blocking forms are diverse, such as PD-1 inhibitors with immunosuppressive factors can strengthening prevent tumor growth and metastasis, and it with other killer cells can also enhance the anti-tumor effects achieved. The objective response of ICPis is different in each type of cancers. In addition, ICPis is usually accompanied with the risk of immune-related adverse events (irAEs) such as dermatitis and enterocolitis, if the treatment is not adjusted in time, it may be life-threatening. This indicates that patients need to be monitored in real time. Therefore, developing reliable biomarkers to predict patients with irAEs remains a challenge. But the structure of the human body is complicated. After the drug enters the body, it is affected by various factors such as bacterial flora and virus in the body. Even though PD-1 mono or combination options are increasingly used, response rate and duration of response are still where we to understand. Moreover, the regulatory factors of combined treatment and its downstream signaling pathways need to be further identified. Based on this, combined with relevant clinical practice theory, the PD-1 combination therapy is studied in depth from animal (such as the construction of nude mouse tumor model and zebrafish model), cells (the construction of various cell experimental models), and molecular level, is needed to promote the development of PD-1 as a target molecule for immunotherapy tumors and lay a new cornerstone to improve the quality of life of cancer patients.

Authors' Note

Cheng-Hao Jin and Ying-Hua Luo conceived and designed the review. Yu Zhang and Guang-Ze Mou drafted the manuscript. Tian-Zhu Li and Wan-Ting Xu drew figures of the review. Tong Zhang, Hui Xue, Wen-Bo Zuo, and Yan-Nan Li revise it critically for important intellectual content. All authors have read and approved the final version of the manuscript. Yu Zhang and Guang-Ze Mou contributed equally to this work. The authors declared no animal and human studies of the research.

Declaration of Conflicting Interests

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