

Current Program in Anti-tumor Immunotherapy: Targeting PD-1/PD-L1 Immune Checkpoint

Xinyuan Gao^{1, †}, Yulan Nie^{2, †, *}, Yuhao Yang^{3, †}

¹College of Biological Science and Medical Engineering, Donghua University, Shanghai, 201620, China

²Department of Biology and Pharmacy, School of Chemistry and Chemical Engineering, Chongqing University of Science and Technology, Chongqing, 401331, China

³College of Pharmacy, Xuzhou Medical University, Xuzhou, 221004, China

[†]These authors contributed equally author

* Corresponding Author Email: nieyulan@cqust.edu.cn

Abstract. There was extensive ongoing research on immune checkpoint inhibitors for immunotherapy over the past few years. Blocking the immune checkpoint therapy can improve the immune response to tumor cells by inhibiting the binding of PD-1/PD-L1. At present, Programmed Death-1 (PD-1), Programmed Death-Ligand-1 (PD-L1), and CTLA-4 (short for Cytotoxic T lymphocyte-associated Antigen-4) are the commonest inhibitory checkpoints. They have been extensively studied and discovered to inhibit anti-tumor immune responses in solid tumors and checkpoint pathways. PD-1 is a primary T lymphocyte inhibitor receptor. The highly selective expression of PD-L1 provides a target for tumor immunotherapy. It enables immunoblockade therapy against PD-1/PD-L1 checkpoint. Targeted medications are a successful kind of therapy. Immune checkpoint-targeting medications have been effective in treating cancer. In the case of cancer, doctors often activate immune control pathways to suppress new anti-tumor immune responses. Our team thoroughly analyzed the PD-1/PD-L1 immune control pathway involved in cancer immunotherapy in this study. And we make an effort to explain their causes as well as the current therapeutic methods. We also outlined the current role of targeted drugs in the clinical therapeutic management of patients with cancer and reviewed the possible future research directions.

Keywords: Cancer, PD-1, PD-L1, neoplasm, immunotherapy, checkpoint therapy, inhibitory pathways, target combination therapies.

1. Introduction

Immunosuppressive molecules include Programmed Death-1 and Programmed Deathligand-1. Previous studies showed that when it comes to tumors, persistent inflammation, and other pathological circumstances. PD-1/PD-L1 inhibit T cells, cause treatment resistance, and aid in immune escape. This study describes the status of the PD-1/PD-L1 in immunotherapy from the viewpoints of PD-1/PD-L1 structure, mediated immune regulation and therapeutic approaches.

2. PD-1/ PD-L1 Composition

PD-1 is a transmembrane glycoprotein of type I origin. Among them, an extracellular region presents an IgV-like domain. There are 4 N-linked glycosylation sites, and the IgV-like domain is closely associated with the induction of negative immune responses. Furthermore, there are immunoreceptor tyrosine inhibitory gene sequences and switch gene sequences in the cytoplasmic region, which can cause PD-1-mediated immunosuppression when combined with phosphatase[1]. PD-L1 has the strongest regulatory effect among the two ligands of PD-1. It is mainly a transmembrane protein composed of 290 amino acid subunits. It has two immunoglobulin constant regions in the extracellular segment, namely Igc. PD-L1 contains an intracellular region. The intracellular region is mainly composed of amino acids. PD-L1 is most often expressed in

hematopoietic cells which can effectively reduce the incidence of excessive immune response and effectively avoid self-injury of the body[2].



Figure 1. (a) Structure of PD1. (b) Structure of PD1/PDL1 complex.

Created with pubs.rsc.org

3. PD-1/PD-L1 mechanism immunomodulation

Inhibiting immune response, controlling immunological tolerance, and preserving immune balance are PD-1/ PD-L1 molecules in immunity (Figure 2) [3]

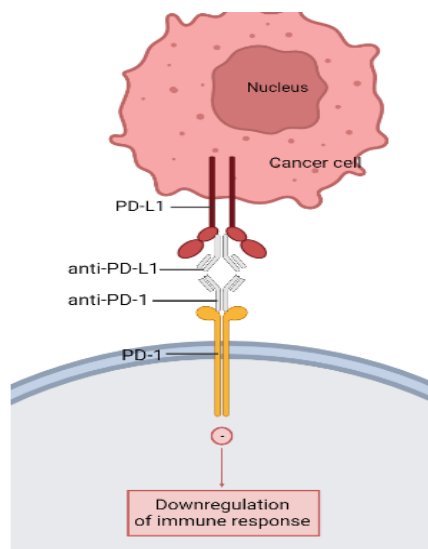


Figure 2. PD1/PD-L1 Binding Mechanism

Created with BioRender.com

By blocking the PI3K/ATK pathway and up-regulating p27 kipl to encourage increasing effector T cell numbers, the PD-1 molecule reduces the production of anti-apoptotic gene Bcl-XL, hence preventing T cell growth[4]. PD-1/PD-L1 also inhibits IL-2, which is necessary for T-cell activation, by blocking protein kinase C phosphorylation. This suppresses effector T cell proliferation. The analysis found that Src homology region protein tyrosine (SHP1/2) recruitment has the ability to reduce the required for activating T lymphocytes and block the phosphorylation of the tyrosine kinase ZAP70. Additionally, SHP1/2 phosphorylation reduces the production of the transcription factor BCL-6 and lowers CD4+ T cells' capacity to differentiate toward polyclonal T lymphocytes.

Under normal physiological conditions, PD-1/PD-L1 can trigger many inhibitory signals and pathways, inhibit T cell activation, survival, and migration, cause effector T cells to die, maintain immune balance, and prevent the development of autoimmune diseases. In immune meningitis and

immunological diabetes, autoimmune disorders are connected with loss of PD-1/PD-L1 signaling. Activating PD-1/PD-L1 effectively controls monoclonal antibody's potency, prevents the hyperplasia of activated T cells, and prevents ongoing tissue damage in immunity to infection and transplantation.

3.1. Intervention of Tregs

Forkhead-like transcription factor (FOXP3), a key regulator of Treg formation and function, has a particular phenotype that is unique to Treg. Treg can be categorized into thymic-derived, peripheral-derived, and ex-vivo-induced Treg, depending on where the root of the cell came from. The development and operation of Tregs are significantly influenced by the particular FOXP3 expression. PD-1 and PD-L1 can boost FOXP3 to support its inhibitory activity and influence the growth of Tregs.

A polyclonal to PD-1/PD-L1 can impact Treg development, multiplication, and functionality. By inhibiting FOXP3 expression on the surface of Tregs, anti-PD-1/PD-L1 lessens Tregs' immunosuppressive effects when administered to mononuclear cells from melanoma patients.

As opposed to that, inhibiting the connection can increase T cell receptor signaling, activate Tregs and improve their inhibitory function. Zhang Y [5] indicated that the infiltration of active Tregs in some patients with advanced gastric cancer was significantly enhanced after anti-PD-1 antibody treatment. This suggested that using an anti-PD-1/PD-L1 antibody may help Tregs proliferate.

3.2. Intervention of TILs

Lymphocyte infiltration can be observed in tumor tissue, and these lymphocytes are called tumor infiltrated lymphocytes (TILs). TILs are the main immune cells that play an immune-killing effect on tumors, and they primarily perform tumor immunity[6]. However, in tumor cells and macrophages under tissue hypoxia, hypoxia-inducible factor-1 can increase PD-L1 expression. Additionally, tumorigenic stimuli can induce PD-L1 expression. PD-L1-expressing cells adhere to T lymphocytes to prevent TILs from multiplying and secreting cytokines. This results in tumor cells evading immune cell surveillance, forming immunological escape, and lessening the TILs. Anti-PD-1/PD-L1 can release TIL, which promotes tumor regression[7]. To detect and destroy tumor cells, CD8+ cytotoxic T lymphocytes are activated when the PD-L1/PD-1 axis is blocked.

3.3. Intervention in TAMs

According to activation mechanisms, released cytokines, and cell morphologies, macrophages infiltrating the tumor microenvironment are referred to as TAMs (tumor-associated macrophages). These macrophages are separated into M2-type macrophages and M1-type macrophages that are selectively activated. M2-type TAMs can inhibit tumor-infiltrating lymphocyte-mediated antitumor immunity and secrete tumor-promoting products, so they are considered to have more important tumor-promoting effects. Tumors can induce overexpression of TAMs by producing IL-10 and macrophage-colony stimulating factors in the microenvironment, thereby impeding normal antigen presentation and tumor immunological reactions. PD-1/PD-L1 antibody enhances macrophage phagocytosis rates. PD-1/PD-L1 changes M1 macrophages into M2.

4. Anti-tumor drugs

Anti-tumor drugs (also can call agents) typically approached from four angles: directly affecting DNA, interfering with nucleic acid biosynthesis, inhibiting protein synthesis and function, and targeting anti-tumor agents. Preclinical and clinical studies demonstrate the effectiveness of novel immune checkpoint-targeting medications in the cancer remedy, and checkpoint therapy improves survival for a more significant percentage of cancer patients. Also, to follow the topic, we here will emphasize drugs that play different roles in clinical therapy development and targeting treatment at the PD-1 or the PD-L1 checkpoint.

4.1. Targeted anti-tumor agents

Targeted anti-tumor agents aim at Tyrosine Kinase(TK). TK can increase the number of white blood cells (WBC), and the Tyrosine kinase inhibitors have good inhibitory activity against PKC. It strengthens the activities of inhibiting PKC and then inhibiting Bcl-Abl protein kinase. Most PD-1/PD-L1 MAbs (short form of monoclonal antibodies) distribute in little number, and most of them are distributed in blood vessels and intercellular space, with a long half-life and steady-state time. Metabolic elimination modes include non-specific elimination (such as proteolytic activity) and target-mediated elimination (including target binding, proteolytic action, and internalization).

PD-1 plays a vital character in the progress of tumor and immune evasive tumor cells. PD-1 promotes the apoptosis of T lymphocytes through PD-1-dependent and independent mechanisms. PD-1 also results in tumor cells in the immune evacuation. Besides, PD-1 inhibitors can block up this immune escape effect, promote the function recovery of T lymphocytes, and enable T lymphocytes to play a normal role in killing tumor cells. [4] [7] [11]

4.1.1 Monoclonal antibody targets PD-1

Pembrolizumab, Nivolumab, Cemiplimab, Toripalimab, and Tislelizumab acting on target PD-1 were all humanized or completely human immunoglobulin G (4IgG4) antibodies.^[7]

Pembrolizumab binds mainly to the C' loop of PD-1, used to treat metastatic melanoma (MM), surgical resection to a high-danger melanoma (adjuvant context), metastatic NSCLC (the abbreviation of non-small-cell lung cancer), HL (short for classic Hodgkin's lymphoma), primary mediastinum.

Nivolumab, which binds to the N-loop, one of the structures of PD-1 checkpoint, is used to treat MM, metastatic NSCLC, and RCC (renal cell carcinoma), head and neck squamous cell carcinoma (SCCHN), classic HL, hepatocellular carcinoma (HCC), MUC (metastatic urothelial carcinoma), MSI-H and MMR type aberrations of colorectal cancer.

Cemiplimab and Toripalimab mainly bind to the FG ring of PD-1, and Cemiplimab mainly attaches to the CC' ring and FG ring of PD-1. It's the treatment of locally advanced CSCC (the abbreviation of cutaneous squamous cell carcinoma) or metastatic CSCC ineligible for surgery or therapeutic radiotherapy.

Tislelizumab is the only one that has been done on the FC ring of PD-1 MAbs with special genetic engineering that can avoid the occurrence of drug resistance. It's also used for the treatment of classic HL and superior urinary tract carcinoma and first-line treatment of advanced squamous NSCLC [4].

4.1.2 Monoclonal antibody targets PD-L1

Avelumab, Durvalumab, and Atezolizumab acting on target PD-L1 are humanized or fully human IgG1 antibodies. The binding epitope region of Avelumab is mainly composed of the C chain, C' chain, F chain, G chain and the CC' ring of PD-L1. In contrast, the binding region of Durvalumab is the CC' ring and N-terminal region of PD-L1. Atezolizumab mainly binds to BC and C of PD-L1 C', C'C" and FG ring residues dominate the binding. [4] [7]

Because the MAbs adverse PD-1/PD-L1 pathway is not metabolized by cytochrome P450 enzyme and other drug metabolism enzymes, drug interactions are less common. Navurlizumab and Palivizumab have broad indications and can be used in melanoma, NSCLC, HL, uroepithelial carcinoma, etc. While Cindillizumab and Carrellizumab are currently approved only for treating relapsed/refractory Hodgkin lymphoma. [4][7] [10]

In 2014, the FDA first approved the PD-1 and its receptor PD-L1 blocker: Pembrolizumab, for cancer therapy. There are currently several drugs in clinical development that target PD-1/PD-L1 interactions. Until now, Nivolumab (Opdivo), Pembrolizumab (Keytruda), and have been approved for clinical application. Atezolizumab (Tecentriq), Avelumab (Bavencio), Durvalumab (Imfinzi) and sevveral other investigational drugs [Table.1] [Table.2] that are not yet in clinical trials are being studied. [1-2]

Table 1. Briefly summarize the completed and ongoing clinical drugs of PD-1

Target	Medicine	Adaptation disease
PD-1	Nivolumab (Opdivo)	To treat patients with unresectable or MM. To treat the wild type of unresectable BRAF V600, or MM. For the treatment of mutational BRAF V600, non-transferable or MM. To treat NSCLC, positive for PD-L1 and progressed after further processing. Combination chemotherapy is an intervention treatment for ter non-squamous/ squamous NSCLC.
	Pembrolizumab (Keytruda)	To treat metastatic NSCLC that is positive for PD-L1 and has progressed after other treatments. For the routine treatment of refractory urothelial carcinoma.
	Cemiplima (Libtayo)	To treat advanced CSCC.
	Tislelizumab	Used in the treatment for classical HL and upper urinary tract carcinoma. Treat the patients with terminal squamous NSCLC on the interventions for the first treatment.

Table 2. Briefly summarize the completed and ongoing clinical drugs of PD-L1

Targ et	Medicine	Adaptation disease
PD-L1	Avelumab (Bavencio)	To treat metastatic Merkel cell carcinoma (MCC). To treat locally terminal or metastatic transitional cell carcinoma.
	Durvaluma b (Imfinzi)	For treatment of locally terminal or MUC. To treat locally advanced NSCLC. It is used in addition to the antiangiogenic drug Axitinib for patients with advanced RCC.
	Atezolizumab (Tecentriq)	For MUC, metastatic NSCLC (single agent and combined with chemotherapy), metastatic SCLC (combined with chemotherapy) chemotherapy, triple-negative metastatic breast cancer (combined Paclitaxel).

4.2. Adagrasib

Adagrasib is one of the most noteworthy drug predictions. Adagrasib is a targeted treatment for cancers with KRASG12C mutation and will be the first such treatment option in colorectal cancer (CRC) patients suffering from this mutation. Common mutations of oncoprotein KRAS variants are traditionally considered to be intractable drug targets for development. A KRAS inhibitor for mutation-positive solid tumors. The first entry of Adagrasib is CRC, and the second entry is NSCLC. Even being second in the market for patients with metastatic NSCLC, Adagrasib is still an effective therapy for the biomarker-defined population making the clinical success.

Jänne and Pasi^[13] et al. evaluated Adagrasib in NSCLC Patients with KRASG12C mutations who had received radiotherapy based on platinum or anti-PD-1 /PD-L1 pathway. David M. Briere with his team [14] assessed five KRASG12C-mutated human mammalian tumor models grown in immunocompromised mice. Determination of the effect of KRASG12C on the expression of genes

key to the tumor cell response to PD-1 inhibition and PD-1 antibodies were also associated with rapid tumor regression in all mice. In addition, they disappeared that the frequency of T cells was increased in tumors treated with PD-1 MABs compared with controls.

5. Other treatments

5.1. Combination of PD-1/PD-L1 inhibitors with other therapy

5.1.1 Molecular targeting drugs

The leading causes of PD-1 or PD-L1 checkpoint obstruction are the insufficient infiltration of T cells as well as the microenvironment causing immunosuppression of tumor.

The issues above can be resolved with Met@Man-MPs(3M) reset TAM. 3M can be used to reduce anti-PD-1 therapeutic resistance. The ability of MAN-MPS to degrade collagen can promote infiltration of CD8⁺T, thereby inhibiting tumor growth, promoting the penetration of antibodies, enhancing the efficacy of combination therapy as well as long-term memory immunity.

Similarly, low-dose apatinib (VEGFR2-TKI) improved hypoxia, boosted CD8⁺ T cell infiltration, decreased TAM recruitment, and decreased serum and tumor TGF levels. A small cohort of patients who were attacked by advanced NSCLC and had received a lot of prior treatment showed excellent anti-cancer efficacy when these two therapies were combined.

Moreover, Wang's team discovered that a 3rd-line therapy for advanced NSCLC, which included the usage of anlotinib capsules with Camrelizumab, substantially lowered serum concentrations of diagnostic markers and cancer tiredness. The effectiveness of that outperforms anlotinib alone [15].

5.1.2 Chemotherapy

Chemotherapy aims to stop cancer cells from generating and replicating DNA. Further, it stimulates tumor-specific T cells, hastens DC maturation, or improves the expression of tumor antigens following cancer cell death.

A more persistent response can be generated by the proper combination of chemotherapy and PD1/PD-L1 inhibitors, especially in cancers that are poorly immunogenic and sensitive to chemotherapy. Nivolumab combined with chemotherapy was found to be superior to chemotherapy in treating gastric/GEJ/oesophageal adenocarcinoma, according to clinical studies. Overall survival or progression-free survival was the trial's primary objectives. Results demonstrated that combining chemotherapy and nivolumab improved OS and PFS significantly.

Anti-PD-1 antibodies combined with cisplatin and pemetrexed to treat MPM were also evaluated by Otsuka's team. There is a significant increase in Foxp3⁺ cells and a decline in MDSCs and vascular irregularities as a consequence of VEGF presentation being suppressed by CDDP and PEM. [16].

The safety of the combination of these two therapies also needs to be carefully considered. One retrospective study analyzed 27 patients with advanced HCC. The primary targets are also OS and PFS. The conclusion is that HAIC, anti-PD-1 antibodies, and TKIs are effective and safe treatments.

5.1.3 Cancer vaccine

Worldwide, HCC continues to be a major factor in cancer-related fatalities. To enhance the prognosis of HCC patients, additional immunological changes and the development of novel treatment approaches are urgently required.

The patient's immune system is stimulated by the tumor vaccination, improving immunogenicity and controlling or eliminating tumors. Immunotherapies for HCC that combine vaccinations and PD-1 immune checkpoint inhibitors have been considered, but their therapeutic efficacy has not yet been determined. According to the results of a recent study, in liver cancer cells, vaccine and PD-1 or PD-L1 inhibitors increased the cytotoxicity of T lymphocytes, resulting in smaller tumor volume [17]. Another trial hypothesized that the synergistic anticancer effects of PD-1 blockade and LMDD-MPFG were achieved by altering TAMs or attenuating T cell suppressive signaling. It is found that

in HCC cells, although LMDD-MPFG re-sensitized tumor-associated T-cells to anti-PD-1 immunotherapy, it enhanced PD-L1 expression [18].

Besides, the immunoregulatory IDO/PD-L1 vaccine used in conjunction with nivolumab and neo-PV-01 combined with PD-1 inhibition are both proved to be effective and safe for metastatic or advanced melanoma. Antigen-specific responses are likely generated after immunization.

5.2. The CRISPR/Cas9 system

To further improve cure rates for cancer development and metastasis, CRISPR/Cas9 technology is being used to correct mutated genes, alter specific genes, and knock in or out certain genes.

As a potential target for GBM treatment, PD-L1 is essential for the immunosuppression, survival, proliferation, and migration of GBM. With CRISPR/Cas9 technology, PD-L1 could be deleted. To increase the specificity and effectiveness of PD-L1 knockdown, an HDR template binding to dual sgRNAs can be employed.

Another study has also been linked to GBM. By repeating the CRISPR-Cas9 system using clustered, regularly spaced short palindromes, EVCAR-T cells which have disrupted PD-1 were created. It was found that these cells could inhibit the growth of EGFRVIII-expressing GBM cells in vitro [19].

Many current studies aim to interdict the PD-1/PD-L1 access enduringly. For this goal, an optically switched CRISPR/Cas9 system was designed. This system used for PD-L1 genome disruption, can effectively activate T cell responses to combat cancer cells and enhance CD8⁺T cell invasion into tumor tissues.

Meanwhile, a nano delivery system based on CRISPR/Cas9 gene editing technology was established to restrain the PD-L1 gene permanently. The edited plasmid was sent to B16F10 cells using the polyamine amine analog AP-PAMAM in the cure of melanoma utilizing this technique.

5.3. CAR-T cell therapy

The ability of human CAR-T cells has been significantly increased by PD-1 inhibition, which may overcome the immune suppression seen in PD-L1⁺ tumors.

Immune checkpoint inhibitors and CAR-T cells were clarified to promote CAR-T cell growth. According to a trial in patients with R/R CD30⁺ lymphoma, the combination demonstrated enhanced effects on CD30 CAR-T therapy with minimal toxicity [20].

Other strategies have also been designed. CAR-T cell has been modified to secrete scFv to block PD-1. These cells enhance the antitumor activity through paracrine and autocrine effects. Since released scFvs remain in the tumor as well as guard CAR-T cells against PD-1 suppression, this approach avoids the side effect of systemic checkpoint inhibition, thus improving safety.

Moreover, a chimeric PD-1/CD28 receptor is formed when the intracellularly domain of PD-1 joins forces with the membrane-associated and cytoskeleton domains of CD28. This receptor interacts with PD-L1 and conveys an activation signal rather than an inhibitory one typically sent by the intracellularly domain of PD-1. Liu's team developed a unique CD19-PD-1/CD28-CAR-T cell line, which is distinct from the prototype CD19-CAR-T cell line. It was found that due to their superior proliferation and cytokine production ability, PD-L1⁺ B-cell lymphoma cells could be fully killed.

6. Conclusions

We still confront a lot of issues and difficulties, despite the fact that many medicines directing at the PD-1/PD-L1 access have made some initial headway in clinical treatment. Some antibody drugs have obvious individual differences. There are restrictions on the illnesses that can be healed with PD-1/PD-L1 antibodies.

Except for cancer cells using PD-1 as camouflage, other normal cells also use PD-1 to avoid being attacked by T cells, and once the PD-1 blocker is active, these normal cells will be targeted as well,

which will cause huge side effects. Therefore, additional measures may be required to lower the frequency and severity of the side events while maintaining the combination's efficacy.

Moreover, even though CAR-T cell immunotherapy is quite safe in comparison to other modalities, it is still necessary to research ways to increase T-cell survival. Although the gene-editing CRISPR/Cas9 technology disclosed in recent years has a wider variety of applications than several conventional therapeutic approaches, it is urgently needed to figure out how to transition from basic research to clinical use.

In order to give the best treatment plan for clinic patients and enhance the practical application effect of clinical application, scientists need to develop more accurate and sensitive detection techniques for tumor identification, efficacy monitoring, and prognosis assessment, etc.

References

- [1] L. Chiew Woon, L. Joycelyn Jie Xin, C. Su Pin, Nivolumab for the treatment of hepatocellular carcinoma, *Expert Opin Biol Ther* **20**(7) 687-693. (2020)
- [2] Liu Y, Yang S, Ren S, the Research progress of PD-1/PD-L1 inhibitors, *Chinese Journal of Medical Frontiers (Electronic Edition)* 10(7) 8-16. (2018)
- [3] Fu Y, Zong B, Li X, Shen L, PD - 1 / PDL - 1 in the diagnosis and treatment of gastric cancer research progress, the Chinese and foreign medical research 19 (9) 194-196. (2021).
- [4] Wang X, Shi Y, Yue F, Research progress and clinical application of PD-1/PD-L1 antibody blocking drugs, *Journal of Henan Normal University (Natural Science Edition)* 49(2) 87-92. (2021)
- [5] Zhang Y, Liu X, Wang J, Cheng M, molecular modeling studies of PD-1/PD-L1 small-molecule immunosuppressants, *Chinese Journal of Medicinal Chemistry* 30(4)209-216. (2020)
- [6] Cheng F, Zheng W, An L, Sun Y, Effect of high-dose recombinant human α -2B interferon on the expression of LDH, TIL and PD-L1 in patients with advanced melanoma, *Chinese Journal of Integrated Traditional and Western Medicine Dermatology* 20(1) 27-31. (2021)
- [7] Lin Y, Zhou X, Lin W, Wang X, Changes of PD-1/PD-L1 and related immune cells in peripheral blood and tumor tissues of patients with cervical squamous cell carcinoma and its clinical significance, *Chinese Journal of Cancer Biotherapy* 29(4) 332-337. (2022)
- [8] Y. Han, D. Liu, L. Li, PD-1/PD-L1 pathway: current researches in cancer, *Am J Cancer Res* **10**(3) 727-742. (2020)
- [9] Y. Zhuang, C. Liu, J. Liu, G. Li, Resistance Mechanism of PD-1/PD-L1 Blockade in the Cancer-Immunity Cycle, *Onco Targets Ther* **13** 83-94. (2020)
- [10] M. Ikeda, Y. Koh, S. Teraoka, K. Sato, J. Oyanagi, A. Hayata, N. Tokudome, H. Akamatsu, Y. Ozawa, K. Endo, M. Higuchi, M. Nakanishi, H. Ueda, N. Yamamoto, Longitudinal Evaluation of PD-L1 Expression on Circulating Tumor Cells in Non-Small Cell Lung Cancer Patients Treated with Nivolumab, *Cancers (Basel)* **13**(10). (2021)
- [11] T. E. Keenan, K. P. Burke, E. M. Van Allen, Genomic correlates of response to immune checkpoint blockade, *Nat Med* **25**(3) 389-402. (2019)
- [12] B. Wen, L. Zhao, Y. Wang, C. Qiu, Z. Xu, K. Huang, H. Zhu, Z. Li, H. Li, Nanobodies targeting the interaction interface of programmed death receptor 1 (PD-1)/PD-1 ligand 1 (PD-1/PD-L1), *Prep Biochem Biotechnol* **50**(3) 252-259. (2020)
- [13] P. A. Jänne, G. J. Riely, S. M. Gadgeel, R. S. Heist, S. I. Ou, J. M. Pacheco, M. L. Johnson, J. K. Sabari, K. Leventakos, E. Yau, L. Bazhenova, M. V. Negrao, N. A. Pennell, J. Zhang, K. Anderes, H. Der-Torossian, T. Kheoh, K. Velastegui, X. Yan, J. G. Christensen, R. C. Chao, A. I. Spira, Adagrasib in Non-Small-Cell Lung Cancer Harboring a KRAS(G12C) Mutation, *N Engl J Med* **387**(2) 120-131. (2022)
- [14] D. M. Briere, S. Li, A. Calinisan, N. Sudhakar, R. Aranda, L. Hargis, D. H. Peng, J. Deng, L. D. Engstrom, J. Hallin, S. Gatto, J. Fernandez-Banet, A. Pavlicek, K. K. Wong, J. G. Christensen, P. Olson, The KRAS(G12C) Inhibitor MRTX849 Reconditions the Tumor Immune Microenvironment and Sensitizes Tumors to Checkpoint Inhibitor Therapy, *Mol Cancer Ther* **20**(6) 975-985. (2021)

- [15] Y. Wang, X. Shi, Q. Qi, B. Ye, Z. Zou, Safety of Anlotinib Capsules Combined with PD-1 Inhibitor Camrelizumab in the Third-Line Treatment of Advanced Non-Small-Cell Lung Cancer and Their Effect on Serum Tumor Markers, *J Healthc Eng* **2021** 2338800. (2021)
- [16] K. Otsuka, A. Mitsuhashi, H. Goto, M. Hanibuchi, K. Koyama, H. Ogawa, H. Ogino, A. Saijo, H. Kozai, H. Yoneda, M. Tobiume, M. Kishuku, K. Ishizawa, Y. Nishioka, Anti-PD-1 antibody combined with chemotherapy suppresses the growth of mesothelioma by reducing myeloid-derived suppressor cells, *Lung Cancer* **146** 86-96. (2020)
- [17] C. F. Teng, T. Wang, F. Y. Shih, W. C. Shyu, L. B. Jeng, Therapeutic efficacy of dendritic cell vaccine combined with programmed death 1 inhibitor for hepatocellular carcinoma, *J Gastroenterol Hepatol* **36**(7) 1988-1996. (2021)
- [18] G. Xu, D. Feng, Y. Yao, P. Li, H. Sun, H. Yang, C. Li, R. Jiang, B. Sun, Y. Chen, Listeria-based hepatocellular carcinoma vaccine facilitates anti-PD-1 therapy by regulating macrophage polarization, *Oncogene* **39**(7) 1429-1444. (2020)
- [19] T. Nakazawa, A. Natsume, F. Nishimura, T. Morimoto, R. Matsuda, M. Nakamura, S. Yamada, I. Nakagawa, Y. Motoyama, Y. S. Park, T. Tsujimura, T. Wakabayashi, H. Nakase, Effect of CRISPR/Cas9-Mediated PD-1-Disrupted Primary Human Third-Generation CAR-T Cells Targeting EGFRvIII on In Vitro Human Glioblastoma Cell Growth, *Cells* **9**(4). (2020)
- [20] W. Sang, X. Wang, H. Geng, T. Li, D. Li, B. Zhang, Y. Zhou, X. Song, C. Sun, D. Yan, D. Li, Z. Li, C. Li, K. Xu, Anti-PD-1 Therapy Enhances the Efficacy of CD30-Directed Chimeric Antigen Receptor T Cell Therapy in Patients with Relapsed/Refractory CD30+ Lymphoma, *Front Immunol* **13** 858021. (2022)