Status, Challenges, And Prospects Of PD-1/PD-L1 Antibodies Therapeutic of Triple-Negative Breast Cancer

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Abstract. Breast cancer is a malignant tumor which has the highest incidence in the world, serious threatening to women's health. Surgery and chemotherapy are the general symmetric therapy for breast cancer. However, the worse prognosis is in triple-negative breast cancer (TNBC) compared with other types of breast cancer due to lack of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). The characteristics of low differentiation and high heterogeneity cause the tumor cells to metastasize more easily, resulting in less effect on traditional drug treatment. For now, immune checkpoint inhibitors (ICIs) show greater effect on treating some kinds of cancers by restoring T cells function. But the clinical results in TNBC are still not very satisfactory, some researchers suggest the combination treatment of ICIs with drug or target therapy can provide better effects. This review summarizes the results in clinical trials in both ICI and ICI-chemotherapy combination, describes the possibility explanations, and discuss the challenges and prospects of ICIs in the treatment of TNBC.

Keywords: TNBC, immune checkpoint inhibitor, ICI-chemotherapy combination.

1. Introduction

Data obtained from the WHO, the breast cancer influence approximately 21 million people worldwide, which represents 2.6 percent of the global population of women, especially the mortality rate of TNBC is up to 23 percent. The associated high incidence and mortality of breast cancer has led to it being recognized as a major global health problem, so reducing the incidence of breast cancer has become a global research focus. According to St Gallen molecular classification, breast cancer is mainly divided into Luminal A, Luminal B, HER-2+ and TNBC [1]. The most special type is TNBC, which accounts for about 10% to 15% of breast cancers, leading to fewer choice for treatments [2]. In addition, TNBC is closely related to the mutated status of breast cancer susceptibility gene (BRCA1/2), and more than 20% of TNBC patients are carriers of this mutation. Biologically, TNBC tumors are more aggressive and larger, having a higher tumor grade, and associating with lymph node metastasis. Due to the lack of a clear molecular target, TNBC treatment relies on chemotherapy, mainly anthracycline and taxeme regimens. Although neoadjuvant chemotherapy treats patients with TNBC, it shows a higher clinical response. When immunotherapy has become a more promising anti-tumor treatment method, it seems to bring new hope to TNBC. The main co-inhibitors, CTLA-4 and PD-1, which are mainly involved in preventing T cells from being over-activated in the tumor microenvironment (TME) [3]. These immune checkpoints help tumor cells escape through suppressing their own anti-tumor immune system and preventing T cells from activating in response. ICIs can combine with immune checkpoints and restart the ability of T lymphocytes, which is clearing tumor cells. In recent years, as a new strategy of anti-tumor immunotherapy, ICIs have made important progress in therapeutic many kinds of solid tumors such as melanoma and lung cancer. A number of clinical trials have confirmed that ICIs monoclonal antibody can make the response rate and prognosis of TNBC patients higher, but the effect is still unsatisfactory, as the average effective rate of patients with multi-line therapy is less than 6%. According to clinical trial data, the current application of PD-L1 monoclonal antibody in TNBC is mainly based on combination therapy, such as chemotherapy or anti-angiogenic therapy combined with PD-L1 monoclonal antibody. This review aims to look back the results of previous clinical trial and discuss the future of ICIs in treating TNBC.

2. The characteristics of TNBC

As the most difficult curable cancer, TNBC has many characteristics that lead to the poor efficacy of ordinary treatment. For example, there are more tumor infiltrating lymphocytes (TILs) in TNBC, which means that its metastatic and spread is faster at a large extent. Secondly, TNBC also has PD-L1, which can interact with PD-1 on CTL, inhibiting the function of immune cell to cause immune escape and exacerbate cancer spread. However, more TILs indicate inflammation in the tumour microenvironment, which make the TNBC is treatable with immunotherapy. In addition, the presence of highly expressed of PD-L1 provides a direct target for immunotherapy. Finally, TNBC has much more non-synonymous mutations that cause its cancer cells to produce tumour-specific neoantigens that active neoantigens-specific CTL to kill cancer cells [3]. This process can also be proved by prospective efficacy of ICIs. Therefore, the TNBC includes three classifications, immune-desert (cluster 1), innate immune-inactivated (cluster 2) and immune-inflamed (cluster 3), according to its tumor microenvironment (TME) of immune escape. The number of immune cells in cluster 1 is poor, which means it has lower immune cells infiltration. The immune escape in cluster 2 might be caused by inactivation of immunity and lower tumor antigen burden. The characteristics in Cluster 3 is excessive infiltration of all kinds of immune cells, which overexpression of immune checkpoints in tumor cells may lead to immune escape [3]. As a result, the third cluster tumors may respond better to ICIs.

3. Immune checkpoint inhibitors (ICIs) treatment of TNBC

Immune checkpoint is a regulatory mechanism within the autoimmune system, which play a key function in the immune cells to clear pathogens and cancer cells to distinguish between self and non-self. A variety of immune checkpoint proteins express on the immune cells to transmit stimulation or inhibition signals in the process of immune to achieve the effect of balanced immune response. Immune checkpoint can be divided into two different types: active and negatively regulated. Negatively regulated immune checkpoints can inhibit immune cell activity and prevent autoimmune cells from overreacting and causing self-damage. For example, the functions of immune checkpoints are inhibition of T cells activation. T cells are activated through the recognition of TCR and antigen-presenting major histocompatibility complex (MHC), costimulatory signaling and cytokine receptors [3]. The binding of costimulatory molecular of CD28 and B7-1/B7-2 help the activation of T cell. The first immune negatively checkpoints discovered on T cell was CTLA-4, which can limit the availability of CD28 [3].

On T cells, there is high expression of PD-1, which interacted with PD-L1 of tumour cells or other tumour stromal cells, inhibiting the killing activity of T lymphocytes and promotes the apoptosis of T lymphocyte to suppress the antitumor effect of T lymphocytes [2].

At present, ICIs is the most effective immunotherapy for cancer treatment. PD-L1 monoclonal antibody therapy can regulate the activity of T cells in the TME and restore the anti-tumor property of CTL cells through downregulation of the PD-1/PD-L1 signaling pathway, enhancement of T cells infiltration of tumors and inhibition of T cells apoptosis [2]. Several recent clinical trials have demonstrated some benefit of ICIs in TNBC therapy.

3.1. ICIs monotherapy

In clinical trials such as KEYMAT-012 study of the PD-1 inhibitor Pembrolizumab showed that the ORR of 32 pretreated and treated PD-L1-positive mTNBC patients was 18.5% [3]. However, a larger study of KEYNOTE-086 found 5.3% ORR in 170 patients with pretreatment without PD-L1 selection, and notably an ORR of 21.4% in 84 treated patients with PD-L1 expression [4]. These two experimental data shows that patients with significant treatment effect of ICIs monotherapy are all PD-L1 positive TNBC cells. But not all TNBC cells are PD-L1 positive, there is study shows that only 46% of TNBC cells are PD-L1 positive. Combination therapy maybe a possible strategy for TNBC patients with PD-L1 negative.

3.2. ICIs and chemotherapy combination

Due to the limited effect of PD-L1 monoclonal antibody therapy on patients, according to the current clinical trial data, it is mainly used in combination with chemotherapy, which can increase the expression of PD-L1 on TNBC cells. SAMANTA D [5] reported that treated TNBC cells of human and mouse with gemcitabine, carboplatin, doxorubicin, or Taxol significantly increased the PD-L1 expression, which helped to enhance the killing effect of PD-L1 monoclonal antibody on tumors.

Chemotherapy is capable of inhibiting the glycolysis of tumor cells, reducing the level of lactic acid [6], and enhancing anti-tumor immunity. Lactic acid can mediate tumor immunosuppression and inhibit the proliferation and activity of CTL. Lactic acid is also capable of inhibition IFN-γ, perforin, granzyme secretion and NKG2D receptor expression on natural killer (NK) cells. The interaction between PD-1 and PD-L1 can inhibit the DCs maturation, activation, degradation, and antigen presentation, promote the myeloid-derived suppressor cells (MDSCs) expansion and tumor associated macrophage (TAM) differentiation into M2 type. CAO B [7]. found that treating breast cancer cells with Adriamycin could significantly inhibit their glycolysis in vitro experiments. Inhibition of lactic acid can increase PD-L1 expression by activating GPR81 expression in tumor cells. The efficacy of PD-1 monoclonal antibody can also be improved by blocking the production of lactic acid. On the contrary, chemotherapy resistant TNBC cell lines showed enhanced glycolysis, increased glucose uptake, and upregulated lactic acid production. Treatment with PD-L1 monoclonal antibody can weaken tumor glycolysis, reduce lactic acid level, reverse chemotherapy resistance, restore glucose utilization by T cells, and improve T cell hyporegiactivity [3].

Indeed, the synergistic mechanisms combined chemotherapy and PD-L1 inhibitors are very complex. Sometimes chemotherapy may inhibit the activity of cytotoxic T cells and reduce the efficiency of PD-L1 monoclonal antibody (MAb), whereas some chemotherapy drugs may enhance the efficacy of PD-L1 monoclonal antibody by regulating the tumor immune microenvironment. In addition, PD-L1 MAb can enhance chemotherapy to induce apoptosis of tumor cells by activating anti-tumor immunity. The I-SPY 2 trial concludes patients who have stage II-III TNBC. This trial showed that when adding pembrolizumab to neoadjuvant paclitaxel, the estimated pathological complete response (pCR) rate increase triple from 22% to 60% [8]. Also, another trail the IMpassion130, atezolizumab combined with albumin-paclitaxel resulted in extension of PFS in patients with metastatic TNBC compared to albumin-paclitaxel monotherapy (7.2 months vs 5.5 months) [3]. These clinical results proved that ICIs of PD-L1 combined with chemotherapy has better therapeutic effect, mainly because chemotherapy could increase TILs and the PD-L1 expression on TNBC cells, which help enhance the killing effect of PD-L1 monoclonal antibody on tumors.

3.3. ICIs and targeting therapy combination

The potential combination of ICIs and other targeted therapy will possibly become the main treatment for TNBC in the future. Poly ADP-ribose polymerase (PARP) protein can participate in the editing and repair of DNA damage in some HER2-negative tumor cells, while TNBC cells may have functional deletion mutations of DNA repair pathways, such as BRCA 1/2 [9]. PARP inhibitors can bind to PARP to block the replication fork of DNA, resulting in the disconnection of the DNA double-stranded structure, making tumor cells unable to complete gene replication [9]. The study also found that PARP inhibitors can also participate in transcription; apoptosis and immune regulation, possibly enhancing the expression of PD-L1 in TNBC cells to become a potential target. Another target is mitogen-activated protein kinase (MAPK), involving in controlling the survival, proliferation, and differentiation of cells [10]. MAPK signal cascades are often misaligned in TNBC. Atezolizumab in combination with Osimertinib, a MAPK inhibitor, in untreated patients with advanced and mTNBC (n = 63) had an ORR of 31.7% in Phase II clinical trial. The ORR of PD-L1 positive subgroup was 39% in comparison with 19% in the PD-L1-negative subgroup [10].

Table 1. Clinical trials of ICIs treatment in TNBC

Clinical trial	Biomarker	Regimen	Number of subjects	Activity results (ORR, %)
PD-1/PD-L1 monotherapy				
NCT01848834 (KEYNOTE-012)	PD-L1+	Pembrolizumab	32	18.5%
NCT02447003 (KEYNOTE-086B)	PD-L1+	Pembrolizumab	84	21.4%
NCT02447003 (KEYNOTE-086A)	PD-L1+	Pembrolizumab	105	5.7%
	PD-L1-	Pembrolizumab	64	4.7%
NCT01772004 (JAVELIN)	PD-L1+	Avelumab	9	22.2%
	PD-L1-	Avelumab	39	2.6%
Chemotherapy combination trials				
NCT01633970	PD-L1+/-	Atezolizumab+ Nab-Paclitaxel	33	39.45%
NCT02425891 (IMpassion130)	PD-L1+/-	Atezolizumab+ Nab-Paclitaxel	902	56.0%
NCT02513472 (ENHANCE-1)	PD-L1+/-	Pembrolizumab+ denibulin	106	26.4%
NCT03125902 (IMpassion131)	PD-L1+/-	Atezolizumab+ Paclitaxel	651	PFS:5.68 months
NCT02819518 (KEYNOTE-355)	PD-L1+/-	Pembrolizumab+ chemotherapy	847	PFS:9.7 months
Target combination trials				
PARP inhibitors				
NCT02734004 (MEDIOLA)	Germline BRCAm	Olaparib+ durvalumab	17	58.8%
NCT02657889 (TOPACIO/ KEYNOTE-162)	PD-L1+/-	Niraparib+ pembrolizumab	55	21%
	BRCA+/-		15	47%
	BRCA-		27	11%
MEK inhibitor				
NCT02322814 (COLET)	PD-L1+/-	Nab-paclitaxel vs paclitaxel + cobimetinib +atezolizumab	90	34.4%

4. Conclusion

TNBC, as the most difficult curable breast cancer, its data in clinical trials demonstrates that ICIs successfully improve the poor prognosis of TNBC. However, it should be noted that in the process of summarizing different clinical practices, the heterogeneity in the cell characteristics of TNBC for different therapeutic strategies of ICIs monotherapy or combination therapy. The heterogeneity characteristics on TNBC cells also make the maximum effects of ICIs treatment. On the other hand, it was found that even with the combination treatment of ICIs, the therapeutic effect of TNBC was still not satisfactory as expected. Therefore, in the follow-up research process, it is also necessary to analyze the tumor microenvironment of TNBC in a variety of ways, to enhance the role of ICIs. For example, the immune-microenvironment characteristics of TNBC were analyzed more comprehensively by multiple fluorescence staining, single cell sequencing and other technologies. To find the time when ICIs can be used as maximum effect, searching for the main molecules that can inhibit CTL cell activity in the TNBC immune microenvironment. Studying how specific chemotherapy can regulate immune microenvironment of tumor cells, to clarify the specific

mechanism of action in combination of PD-(L)1 and chemotherapy. Finally, single cell sequencing and multiple fluorescent staining can also be used to find new targets on TNBC cells, providing a new direction for treatment.

References

[1] Zarean Shahraki, Saba et al. “Time-related survival prediction in molecular subtypes of breast cancer using time-to-event deep-learning-based models.” Frontiers in oncologyvol. 13 1147604. 5 Jun. 2023, doi:10.3389/fonc.2023.1147604

[2] Jian Zheng. "Expression of PD-L1 in triple-negative breast cancer microenvironment and its regulatory mechanism by thymosin α1 (Ridaxin) ".2022. doi:10.27652/d.cnki.gzyku.2021.000326

[3] Ba, Hongping et al. “Antitumor effect of CAR-T cells targeting transmembrane tumor necrosis factor alpha combined with PD-1 mAb on breast cancers.” Journal for immunetherapy of cancer vol. 11,1 (2023): e003837. doi:10.1136/jitc-2021-003837

[4] Loi, Sherene et al. “Association Between Biomarkers and Clinical Outcomes of Pembrolizumab Monotherapy in Patients With Metastatic Triple-Negative Breast Cancer: KEYNOTE-086 Exploratory Analysis.” JCO precision oncology vol. 7 (2023): e2200317. doi:10.1200/PO.22.00317

[5] Samanta D, Park Y, Ni X, et al. Chemotherapy induces enrichment of CD47+/CD73+/PDL1+immune evasive triple-negative breast cancer cells. Proc Natl Acad Sci U S A. 2018;115(6):E1239-E1248. doi:10.1073/pnas.1718197115

[6] Zidi O, Souai N, Raies H, et al. Fecal Metabolic Profiling of Breast Cancer Patients during Neoadjuvant Chemotherapy Reveals Potential Biomarkers. Molecules. 2021;26(8):2266. Published 2021 Apr 14. doi:10.3390/molecules26082266

[7] Cao B, Li M, Zha W, et al. Metabolomic approach to evaluating adriamycin pharmacodynamics and resistance in breast cancer cells. Metabolomics. 2013;9(5):960-973. doi:10.1007/s11306-013-0517-x

[8] Mukhtar, Rita A et al. “Breast Conservation Surgery and Mastectomy Have Similar Locoregional Recurrence After Neoadjuvant Chemotherapy: Results From 1462 Patients on the Prospective, Randomized I-SPY2 Trial.” Annals of surgery vol. 278,3 (2023): 320-327. doi:10.1097/SLA.0000000000005968

[9] Fu, Xiaoyu et al. “Mechanism of PARP inhibitor resistance and potential overcoming strategies.” Genes & diseases vol. 11,1 306-320. 24 Mar. 2023, doi:10.1016/j.gendis.2023.02.014

[10] Zhang, Zhizhu et al. “Immunomodulatory Properties of PI3K/AKT/mTOR and MAPK/MEK/ERK Inhibition Augment Response to Immune Checkpoint Blockade in Melanoma and Triple-Negative Breast Cancer.” International journal of molecular sciences vol. 23,13 7353. 1 Jul. 2022, doi:10.3390/ijms23137353