Clinical significance and prognostic analysis of TILs and PD-L1 expression in non-small cell lung cancer

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Abstract: Since the US FDA approved immunotherapy in 2015, immuno-oncology has completely changed the treatment of non-small cell lung cancer (NSCLC) and has become a new and effective treatment.However, the overall effectiveness of immunotherapy is only 20% to 30%, only a small number of patients can benefit from it, and it also produces a series of toxic side effects.Studies have shown that tumor-infiltrating lymphocytes (TILs) are associated with a variety of tumor outcomes, especially in lung cancer.Therefore, there is still room to improve outcomes for patients with non-small cell lung cancer.Finding efficient and accurate biomarkers to predict and screen cancer patients with good efficacy for PD-1/PD-L1 inhibitor therapy has become an important clinical research hotspot and urgent need.The purpose of this review is to elucidate the clinical significance of TILs and PD-L1 expression in NSCLC and its correlation with prognosis, and to provide clinical reference for patients receiving immunotherapy.

Keywords: NSCLC, TILs, PD-L1, immunotherapy

1. Introduction

Lung cancer is one of the malignant tumors with the fastest increasing morbidity and mortality and the greatest threat to human health and life.Non-small cell lung cancer (NSCLC) is the most common histological type (80% to 85%) and includes squamouscellcarcinoma (SCC) and adenocarcinoma (ADC), large cell lung cancer (LCC), Adenosquamous carcinoma lung cancer (ASC).Current treatments for NSCLC include surgical resection, chemoradiotherapy and targeted therapy.Despite great advances in new drugs and systemic treatments, the 5-year survival rate for patients with advanced NSCLC is only 5%.In recent years, with the research of immunotherapy, immune checkpoint inhibitors (ICIs), as a kind of immunotherapy-based drugs, have shown obvious clinical advantages in the treatment of lung cancer.ICIs mainly enhances the function of immune cells in the human body to achieve the effect of strengthening tumor suppression.At present, ICIs has been widely used in the first-line treatment of metastatic lung cancer.However, studies have shown that the overall effectiveness of this immunotherapy is only between 20% and 30%, and only a very small number of patients show a sustained response to treatment[1] .Due to the development of primary or acquired drug resistance, most lung cancer patients do not respond well to this therapy.At the same time, as an immune-mechanism drug, ICIs is accompanied by a series of immunotoxic reactions, such as checkpoint inhibitory pneumonia (CIP), colitis, pituitaritis, thyroiditis, inflammatory arthritis, etc.[2] .In recent years, it has been found that TILs in tumor microenvironment are associated with the prognosis of NSCLC. Therefore, this article reviews the relationship between TILs in tumor microenvironment and prognosis of NSCLC.

2. Relationship between TILs in tumor microenvironment and prognosis of NSCLC patients

The composition of the tumor microenvironment is complex and typically includes immune cells such as T lymphocytes and B lymphocytes, tumor-associated macrophages (TAM), dendritic cells (DC), natural killer cells (NK), neutrophils and myeloid suppressor cells (MDSC).Interstitial cells, extracellular matrix (ECM), other secretory factors, and blood and lymphatic networks act on heterogeneous cancer cells and constantly cooperate with each other.In addition, there is increasing evidence that the

composition of the tumor microenvironment has an important impact on the development and prognosis of patients with lung cancer.Among them, the TILs mainly exists in the Tumor microenvironment of a group of heterogeneous lymphocytes,It consists of all monocytes (lymphocytes, plasma cells, monocytes, and NK-T cells) scattered in the stromal TILs of the tumor or in the intratumoral TILs located within the tumor [3][4][5].It can affect tumor progression and the success of anticancer treatment through its pro-tumor and anti-tumor effects [6].Studies have shown that TILs has become an immunological biomarker associated with anti-tumor immune response in melanoma, colorectal cancer, and breast cancer[7].In 1987, Kradin and his team first reported the trial of tumor TILs in NSCLC patients, but the results showed no relevant response;In 2018, Ben-Avi et al. evaluated the feasibility of TILs generation in 5 patients with advanced NSCLC who underwent surgery based on the well-established melanoma TILs protocol, and the results showed that there was an objective response[8].In 2021, BenjaminC et al. conducted a study on the safety and effectiveness of autologous TILs combined with opdivo in 20 patients with advanced NSCLC with opdivo monotherapy, and the results showed that TILs treatment became a promising candidate for lung cancer immunotherapy, and the proportion of CD4+TIL in NSCLC was relatively high[9].Existing clinical research data clearly indicate that the expression of TILs in tumor microenvironment not only CD4+TILs subtypes, but also CD8+TILs, CD68+ macrophages and other subtypes is closely related to the prognosis of NSCLC patients. Therefore, therapeutic strategies targeting TILs are expected to expand the scope of immunotherapy.To benefit a wider range of patients.

2.1 Relationship between CD4+TILs and prognosis of NSCLC

CD4+T lymphocytes can currently be divided into the following subtypes: Th1 cells, Th2 cells, Th17 cells, Th22 cells, Th9 cells, and regulatory T cells (Tregs), with different subtypes playing different roles in the immune system.An early review of circulating T cells in patients treated with ipilimumab(anti-CTLA-4) found that an increase in CD4+T cell percentage at later 8-14 weeks was also associated with positive clinical outcomes[10] As an important immune cell, CD4+T lymphocytes have attracted much attention in recent years.Researchers who studied melanoma have deeply explored the role of CD4+T cells and found that this type of cell has the ability to kill tumors.Through MHCII class tetramer separation technology, experimental results show that antigen-specific CD4+T cells (NY-ESO-1,MAGE-A3) have significant single-cell toxicity and can directly kill tumor cells pretreated with IFN-g or stably enhanced with CIITA transduction[11].In the study of NSCLC, relevant scholars used flow cytometry to detect that TILs in patients with this disease were mainly composed of 13 different immune cell types, among which T cells dominated and CD4+T cells had the largest number (26%), and CD4+TILs were closely related to the prognosis of NSCLC.Draghi et al. further demonstrated that tumor-specific CD4+ TILs can kill their own cancer cells via MHCII class (MHC-II), independent of functional MHCI class (MHC-I) antigen presentation or IFNg signaling.This may have potential and far-reaching implications for the optimization of TILs therapy and immunotherapy[12].

However, a meta-analysis conducted by Chen B et al. indicated that NSCLC patients with a high density of CD4+ TILs in the tumor nest of intratumor lymphocytes (TN) will have better overall survival (OS) (HR = 0.88;95% ci, 0.77-0.99;p<0.001) and disease-specific survival (DSS) (HR = 0.75;95% ci, 0.52 to 0.98;p<0.001).In tumor stroma (TS), high CD4+ TILs density predicted patients with better DSS for NSCLC treatment, but there was no significant correlation between CD4+ TILs and OS in these patients [13].On the one hand, when CD4+ T cells are activated, cytotoxic T lymphocytes (CTLS) can activate CD4+ T cells through various mechanisms to maintain and enhance the anti-tumor response of CTLS in tumors.On the other hand, regulatory T cells, another subtype of CD4+T, may have the opposite effect on tumor immunity due to their immunosuppressive properties, suggesting that CD4+TILs in tumor stromal lymphocytes (TS) have a complex effect on tumor immunity.Studies have also shown that CD4+ lymphocytes are multifunctional cells that can differentiate into helper phenotypes of CD8+ cytotoxic T cells or regulatory T cells that inhibit cytotoxic T cells.It may play a dual role of inhibition or anti-tumor immunity in the tumor microenvironment.This may be determined by the balance of the tumor microenvironment and the individual nature.With the continuous efforts of researchers, it has been shown that the expression of CD4+TILs in the tumor periphery, especially in the intratumoral matrix, is closely related to the poor prognosis of patients with NSCLC after surgery[14].Therefore, it is necessary to further explore the relationship between CD4+TILs and prognosis of NSCLC.

2.2 Relationship between CD8+TILs and prognosis of NSCLC

In NSCLC, CD8+T cells are the second most abundant T cell population after CD4+T cells, and are most strongly associated with good prognosis.Early relevant studies pointed out that TILs, especially

CD8+TILs, are associated with better survival rates, especially in NSCLC[15].Yang H et al. conducted a retrospective study to investigate the effect of PD-L1 expression and CD8+TILs density on the prognosis of 178 patients after NSCLC resection.According to the expression of PD-L1 and the density of CD4+TILs, they were divided into four groups. Among them, patients with PD-L1 +/CD8high had the longest survival (P = 0.012), and patients with PD-L1 +/ CD8low had the shortest survival (P = 0.025).Whether statistically significant or not, in different oncogenic driver mutation ODM states (EGFR, KRAS, ALK, ROS1, HER2, and RET states),CD8high TILs showed a better OS trend than CD8 low TILs in each group of patients.It was suggested that CD8 high TILs were independent predictors of overall survival of NSCLC.Therefore, the combination of PDL1 and CD8+ TILs density has more prognostic value in NSCLC than PD-L1 alone[16].The following meta-analysis data by Chen B et al. showed that:In the 45 studies included, which included 11,448 patients, CD8+TILs had a high density,Overall survival (HR = 0.995, 0.99-1.0), progression-free survival (HR = 0.52, 0.34-0.71), disease-free survival (HR = 0.64, 0.43-0.85), relapse-free survival (HR = 0.42,0.18-0.67) and disease-specific survival (HR = 0.56, 0.350.78), and the higher the density of CD8+ TILs in both the cancer nest and stroma, the better the overall survival of NSCLC[13] .Feng Li and his team conducted a meta-analysis to further validate a variety of factors, including treatment methods (including monotherapeutics and combinations of immune checkpoint inhibitors), cancer type (e.g., NSCLC, melanoma, etc.), and the location of CD8+ T cells (i.e., within the tumor, stroma, and aggressive margins).The findings suggest that patients with high CD8+TILs have more clinical benefits that can be used to predict treatment effect, and that the presence of CD8+TILs in the stroma is a stronger biomarker of PFS and OS.High CD8+ TILs are associated with a 48% lower risk of disease progression[17].

Recent studies have shown that the expression level of PD-L1 in NSCLC is up-regulated after concurrent chemoradiotherapy, which provides pathological basis for subsequent consolidation therapy with anti-PD-L1 antibodies (such as durvalumab).Moreover, after concurrent chemoradiotherapy, the density of tumor matrix CD8+TILs will also be increased, which has significant prognostic value in predicting tumor recurrence and death[18].There was a correlation between additional neoadjuvant radiotherapy and increased PDL1 expression and CD8+TILs density.However, when subgroups were made based on tumor histological types, for lung adenoma (LUAD) after neoadjuvant therapy, it was found that the correlation between PD-L1 expression and CD8+TILs density was no longer significant[19].Although CD8+TILs cells are considered to be the best predictor of survival, these results are not associated with immunotherapy[20] .Therefore, it is necessary to further explore the influence of CD8+TILs and PD-L1 expression on patient prognosis and immunotherapy in NSCLC.

2.3 Relationship between CD68+TAM and prognosis of NSCLC

In the Tumor microenvironment, tumor-associated macrophages are the most common tumor-infiltrating immune cells, supporting or inhibiting tumor growth and metastasis.Previous studies have shown that TAM is correlated with the prognosis of various tumors (including liver cancer, stomach cancer, lung cancer, breast cancer, cervical cancer, bladder cancer, pancreatic cancer and rectal cancer).Among them, CD68 was used as the main biomarker to determine the total TAM.CD68 is a transmembrane glycoprotein that is widely expressed in monocyte types such as macrophages, microglia, and osteoclasts.An earlier meta-analysis suggested that CD68 - and CD163-positive tumor-associated macrophages play an important role in the pathogenesis of triple-negative breast cancer.M1-like macrophages express CD68, which plays an important role in defending against tumors by producing free radicals and even causing damage to genomic DNA.Cd68-positive tumor-associated macrophages found in cancer nests or stroma of cancer tissues were significantly correlated with tumor size.However, the role of CD68 in the development of cancer is controversial.Recent studies have found that in invasive bladder cancer, the invasive levels of CD68+TAM and CD68+PD-L1-TAM are significantly reduced after neoadjuvant chemotherapy.In addition, the invasive level of TAM in patients with invasive bladder cancer before treatment has also been shown to be an independent predictor of the prognosis of tumor patients.Recent studies by Zhang et al. indicate that high levels of CD68 expression are positively correlated with tumor grade, tumor size, Ki67 positivity, and other malignant features that indicate a more progressive and aggressive tumor.High expression of CD68 was positively correlated with the abundance of B cells, CD4+ and CD8+T cells, dendritic cells, macrophages and neutrophils.Therefore, CD68 is expected to be a favored immunotherapy target.However, in a study related to cervical cancer, when evaluating CD8 and CD68 immune cells in the tumor microenvironment as internal and external infiltrating factors, it was meaningless to evaluate CD68+ immune cells alone to determine the prognosis, because the presence of these cells was not related to the stage of patients .

At present, there are relatively few studies on the function of CD68+ macrophages in NSCLC,

especially the reports on immunotherapy are scarce.Researchers Lv et al. found that M2 macrophages, characterized by CD68+ (a broad macrophage marker) and CD163+ (an M2-specific marker), were able to suppress the immune response and limit inflammation.MDSCs and CD68+CD163+M2 macrophages are considered biomarkers of treatment response, and the levels of MDSCs and CD68+CD163+M2 macrophages in NSCLC patients are positively correlated during radiotherapy.This suggests that upregulation of these cells may provide useful auxiliary and sensitive biomarkers for the diagnosis of NSCLC, and may predict the treatment response of patients with radiotherapy for NSCLC.It is understood that no immunotherapy drugs specifically targeting CD68+TAM are currently available.However, inhibition of CD68-dependent signaling is a promising therapeutic strategy that can be applied to immunotherapy for multiple tumor types.Therefore, it is necessary to investigate the expression of CD68+TAM in NSCLC and the relationship between CD68+TAM and PD-L1 expression.

3. Relationship between programmed cell death ligands and prognosis of NSCLC

PD-L1 and PD-L1 belong to inhibited co-stimulatory molecules, and their combination can lead to apoptosis of tumor immune cells.Thus, immune escape of tumor cells is induced.It is well known that PD-L1 is expressed on the surface of many cancer tumor cells, which is one of the reasons why tumor cells escape detection in the immune system.In the tumor microenvironment, PD-L1 is the dominant ligand, and upregulated expression usually occurs.In NSCLC, high expression of PD-L1 is often seen as a marker of poor prognosis.The binding of PD-1 on T cells and PD-L1 on tumor cells can negatively regulate the function of T cells, and then promote the immune escape of tumor cells.Clinically, immune checkpoint inhibitors are widely used in the treatment of lung cancer patients as targets and predictors.By blocking the PD-1/PD-L1 pathway, immune checkpoint inhibitors can restore the activity of T cells and enhance the immune system's resistance to tumors .A meta-analysis by Zeng and his team revealed that the overall survival OS and PFS of NSCLC patients treated with PD-1/PD-L1 were significantly higher than those in the chemotherapy group (HR=0.685,95%CI=0.632-0.742,p<0.001).In addition, the expression level of PD-L1 in NSCLC tissues was positively correlated with the efficacy of PD-1/PD-L1 inhibitors.Zhou and colleagues also reached a similar conclusion, that in NSCLC patients with high PD-L1 expression, both OS and PFS were significantly improved.Regarding the correlation between PD-L1 expression and clinical characteristics of NSCLC patients, most studies have found no significant correlation between PD-L1 expression and gender, age, smoking history, and disease characteristics (histological and pathological tumor grade) .

In October 2016, the FDA approved pembrolizumab as a first-line treatment for patients with high PD-L1 expression (≥50%).Following the subsequent approval of the PD-L1 inhibitor Atezolizumab for progression after chemotherapy in patients with advanced NSCLC, in February 2018 Durvalumab was also approved by the FDA for consolidation treatment of stage III NSCLC after chemoradiotherapy.PD-L1 IHC 22C3 pharmDx is currently the only licensed companion diagnostic for NSCLC in China to assist in screening patients eligible for pembrolizumab therapy and optimize treatment regimens to improve its clinical efficacy.The IMpower110 trial compared atezolizumab with platinum-based chemotherapy in patients with advanced NSCLC expressing highly engineered death ligand 1(PD-L1) on tumor cells (TC≥1%) or tumor-infiltrating immune cells (IC≥1%).The results showed that the overall survival (OS) of atezolizumab treatment was significantly longer in NSCLC patients with high PD-L1 expression than chemotherapy.In addition, the Impower131 study revealed that the atezolizumab treatment group performed significantly better than the control group in patients with high PD-L1 expression (OS) (HR value 0.48, 95%CI: 0.29-0.81).However, in a subpopulation of two-thirds of PD-L1 tumor cells with two-thirds of immune cells (about 32% of patients), atezolizumab treatment was less effective (HR 0.72,95%CI:0.52 to 1.00).Impower150 adopted three four-drug regimens of ABCP, BCP and ACP, namely attilizumab + bevacizumab + carboplatin + paclitaxel, bevacizumab + carboplatin + paclitaxel and Attilizumab + carboplatin + paclitaxel, as the drug combination for its phase III study.Based on three clinical trials, IMpower130, IMpower132, and IMpower150, immune-checkpoint inhibitors were found to cause immune-related adverse events (irAEs), often involving the gastrointestinal tract, endocrine glands, skin, and liver.Although there have been breakthroughs in immunotherapy, given that most patients have primary or acquired resistance to existing immunotherapies, the discovery of new immunotherapy targets and modalities both addresses unmet needs and offers opportunities to improve outcomes .

4. Correlation between tumor infiltrating lymphocytes and PD-L1

PD-L1, as an important member of the B7/CD28 co-stimulatory factor superfamily, can be expressed in a variety of tumor-infiltrating immune cells, such as T cells, B cells, NK cells, dendritic cells and macrophages, to regulate appropriate immune response.The expression of PD-L1 in TILs is associated with different degrees of lymphocyte infiltration.TILs increase the expression of PD-L1 by recruiting tumor antigens and releasing cytokines including IFN-γ.After binding to PD-1 receptor, PD-L1 inhibits the transmission of signal to T cells.In addition, anti-apoptotic signals are transmitted to tumor cells, leading to tumor survival and T cell dysfunction.TILs are mainly distributed in the interstitial tissue region around the tumor nests, in which CD8+T cells are the most abundant subgroup.The higher the density of CD8+T cells in the tumor microenvironment, the longer the prognosis.In addition, CD4+ and CD8+TILs ratios were also associated with prognosis in patients with NSCLC.This effect is even more significant in PD-L1-positive NSCLC patients.

According to an earlier study on PD-L1 expression, PD-L1 has been identified as a poor prognostic factor in patients with node-negative NSCLC.In tumors with increased CD8+TILs, the level of PD-L1 expression was independent of other covariates.This paper suggests that CD8+TILs can restore its effect of eliminating tumor cells after successful inhibition of PD-L1 expression, and more critically, individuals who express PD-L1 in tumor cells and have elevated CD8+TILs are associated with poorer survival rates, independent of other factors.This indicates that PD-L1 has the potential to inhibit the activity of CD8+TIL, which obviously has an important effect on the prognosis of tumors.In recent years, Zhang and his team have conducted in-depth studies on the role of PD-L1 and CD8 double-positive TILs in the tumor microenvironment of NSCLC.They divided NSCLC patients into two groups: one group received non-immunotherapy and the other group received anti-PD-1 therapy to block immunosuppression.The results showed that in the non-immunotherapy group, the higher the level of CD8+PD-L1+Tils in tumor tissue was positively correlated with the deterioration of prognosis.Conversely, in the immunotherapy group receiving anti-PD-1 therapy to block immunosuppression, elevated levels of CD8+PD-L1+Tils were positively associated with a good response to anti-PD-1 therapy.According to the consensus of the researchers, PD-L1 and CD8 double-positive TILs may act as a potential indicator that is associated with a high tumor mutation load, pointing to the development of an immunosuppressive tumor microenvironment.In addition, blocking the PD-1 pathway during treatment can also help reduce this immunosuppressive phenomenon and improve the therapeutic effect .

However, in addition to CD8+TILs, other types of TILs such as CD4+ helper/regulatory T cells, CD3+TILs, CD20+B cells, and CD68+ macrophages are also associated with NSCLC prognosis.It is necessary to further explore the correlation between these types of TILs and the expression level of PD-L1.The balance between PD-L1-mediated tumor immune escape and the effective immune response of TILs to cancer may provide a more relevant prognostic and therapeutic tool.

5. Conclusion

TILs in the tumor microenvironment play an important role in the occurrence and development of NSCLC. PD-L1, as a PD-1 ligand, is expressed in a variety of tumor-infiltrating immune cells and also plays a key role in the process of tumor immune escape.Immune checkpoint inhibitors, such as PD-1/PD-L1 axis drugs, have been widely used in the treatment of patients with metastatic NSCLC and have become one of its standard of care.Although immunotherapy for NSCLC has emerged as a promising treatment, achieving a durable clinical response and unprecedented survival rates, it still shows widespread drug resistance and toxic side effects.Therefore, the development and exploration of additional therapeutic targets to achieve more therapeutic efficacy has become critical in immunotherapy for NSCLC.The expression and influence of PD-1 and PD-L1 in NSCLC have not been fully investigated, but they are likely to play a role in the specificity of immune cells.In different types of NSCLC, both interstitial TILs and intratumoral TILs can play an anti-tumor or pro-tumor role, thus affecting the progression and therapeutic effect of NSCLC.Therefore, how to identify relevant indicators or combinations of indicators with prognostic significance remains to be further explored.We believe that immunotherapy-related markers TILs and PD-1/PD-L1 will play an increasingly important role in guiding prognosis and immunotherapy in patients with NSCLC, providing an opportunity to optimize treatment strategies.

References

[1] S D E ,E D W ,L D A , et al.Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology.[J].Journal of the National Comprehensive Cancer Network : JNCCN,2022, 20(5): 497-530.

[2] Suresh K , Naidoo J , Lin C T ,et al.Immune Checkpoint Immunotherapy for Non-Small Cell Lung Cancer[J].Chest, 154(6)[2023-12-11].DOI:10.1016/j.chest.2018.08.1048.

[3] Genova C , Dellepiane C , Carrega P ,et al.Therapeutic Implications of Tumor Microenvironment in Lung Cancer: Focus on Immune Checkpoint Blockade[J].Frontiers in Immunology, 2022, 12.DOI: 10. 3389/ fimmu.2021.799455.

[4] Moreno V , Salazar R , Gruber S B .The prognostic value of TILs in stage III colon cancer must consider sidedness[J].Annals of oncology : official journal of the European Society for Medical Oncology, 2022, 33(11):1094-1096.DOI:10.1016/j.annonc.2022.09.155.

[5] Giatromanolaki A , Anestopoulos I , Panayiotidis M I ,et al.Prognostic Relevance of the Relative Presence of CD4, CD8 and CD20 Expressing Tumor Infiltrating Lymphocytes in Operable Non-small Cell Lung Cancer Patients[J].Anticancer Research, 2021, 41(8):3989-3995.DOI:10.21873/ anticanres. 15196.

[6] Bagaev A, Kotlov N, Nomie K,et al.Conserved pan-cancer microenvironment subtypes predict response to immunotherapy[J].Cancer Cell, 2021.DOI:10.1016/j.ccell.2021.04.014.

[7] T S P,Annegé V,Marco B D, et al.Tumor-infiltrating lymphocytes in the immunotherapy era.[J].Cellular molecular immunology,2020,18(4):842-859.

[8] Zhang L , Chen Y , Wang H ,et al.Massive PD-L1 and CD8 double positive TILs characterize an immunosuppressive microenvironment with high mutational burden in lung cancer[J].Journal for immunotherapy of cancer, 9(6):e002356[2024-04-12].DOI:10.1136/jitc-2021-002356.

[9] Creelan B , Wang C , Teer J ,et al.Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial[J].Nature Medicine, 2021, 27:1410 - 1418.DOI:10.1038/s41591-021-01462-y.

[10] Alexander M ,Kilian W ,Jianda Y , et al.Increases in Absolute Lymphocytes and Circulating CD4+ and CD8+ T Cells Are Associated with Positive Clinical Outcome of Melanoma Patients Treated with Ipilimumab.[J].Clinical cancer research : an official journal of the American Association for Cancer Research,2016,22(19):4848-4858.

[11] Amélie Cachot, Bilous M , Liu Y C ,et al.Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer[J].Science Advances, 2021, 7(9):eabe3348.DOI:10.1126/sciadv.abe3348.

[12] Oliveira G , Stromhaug K , Cieri N ,et al.Landscape of helper and regulatory antitumour CD4~+ T cells in melanoma[J].Nature, 2022(May 19 TN.7910):605.

[13] Chen B , Li H , Liu C ,et al.Prognostic value of the common tumour-infiltrating lymphocyte subtypes for patients with non-small cell lung cancer: A meta-analysis[J].PLoS ONE, 2020, 15(11):e0242173. DOI: 10.1371/journal.pone.0242173.

[14] Giatromanolaki A , Anestopoulos I , Panayiotidis M I ,et al.Prognostic Relevance of the Relative Presence of CD4, CD8 and CD20 Expressing Tumor Infiltrating Lymphocytes in Operable Non-small Cell Lung Cancer Patients[J].Anticancer Research, 2021, 41(8):3989-3995.DOI:10.21873/ anticanres. 15196.

[15] Teng F , Meng X , Wang X ,et al.Expressions of CD8+TILs, PD-L1 and Foxp3+TILs in stage I NSCLC guiding adjuvant chemotherapy decisions[J].Oncotarget, 2016, 7(39):64318-64329.DOI:10. 18632/ oncotarget.11793.

[16] William,Sterlacci,Michael,et al.Expression of PD-L1 Identifies a Subgroup of More Aggressive Non-Small Cell Carcinomas of the Lung.[J].Pathobiology : journal of immunopathology, molecular and cellular biology, 2016.DOI:10.1159/000444804.

[17] Jamiyan T , Kuroda H , Yamaguchi R ,et al.CD68- and CD163-positive tumor-associated macrophages in triple negative cancer of the breast[J].Virchows Archiv, 2020, 477(6).DOI:10. 1007/s00428-020-02855-z.

[18] Yoneda K , Kuwata T , Kanayama M ,et al.Alteration in tumoural PD-L1 expression and stromal CD8-positive tumour-infiltrating lymphocytes after concurrent chemo-radiotherapy for non-small cell lung cancer[J].British Journal of Cancer, 2019, 121(6).DOI:10.1038/s41416-019-0541-3.

[19] Zens P , Bello C , Scherz A ,et al.The effect of neoadjuvant therapy on PD-L1 expression and CD8+lymphocyte density in non-small cell lung cancer[J].Modern Pathology[2024-04-12].DOI: 10. 1038/ s41379-022-01139-y.

[20] A S H , A M F F , A A N ,et al.Surprising impact of stromal TIL's on immunotherapy efficacy in a real-world lung cancer study - ScienceDirect[J].Lung Cancer, 2021.DOI:10.1016/j. lungcan. 2021. 01.013.