

T lymphocytes related biomarkers for predicting immunotherapy efficacy in non-small cell lung cancer (Review)

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Abstract. The immune environment is a determinant of whether patients with cancer can benefit from immunotherapy. Immune checkpoint inhibitors (ICIs) have improved the prognosis of patients with different types of malignancies and have initiated a transformation in tumor therapy. However, some patients cannot achieve a long-term response and several patients even have no response to ICIs therapy. Thus, potential biomarkers that can effectively predict the efficacy of ICIs are essential for their clinical application and for the selection of patients. The accuracy of well-known biomarkers, such as expression of programmed cell death ligand 1 and tumor mutational burden, remains controversial. One of the critical factors for immune responses in the tumor microenvironment is tumor antigen-specific T cell. The density and distribution of tumor-infiltrating lymphocytes, T cells activation and T lymphocytes phenotypes in peripheral blood and serum cytokines have been observed in different types of solid cancer. Although the association with immunotherapy prognosis is in dispute, the prospect of T cell-related biomarkers is encouraged. The present review discusses whether these factors are associated with clinical outcomes of patients with non-small cell lung cancer. The association between several serum cytokines and ICIs therapy efficacy is also discussed.

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6. TCR
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1. Introduction

Lung carcinoma is the most diagnosed cancer type worldwide, and the leading cause of cancer-associated mortality (1). In 2018, 2.1 million new lung carcinoma cases were diagnosed and 1.8 million mortalities were registered, and almost one fifth of the mortalities were by cancer cases (2). Applications of immune checkpoint inhibitors (ICIs) aimed at blocking programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1) and anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) have improved overall prognosis compared with traditional chemotherapy and radiotherapy (3-5). PD-L1 expression is a common biomarker to predict response to ICIs and is detected in patients with advanced non-small cell lung cancer (NSCLC). The positivity of PD-L1 has been associated with high probability of response (6). However, other studies have failed to identify an association between PD-L1 expression and ICIs treatment outcomes (3,7,8). A meta-analysis reported that PD-L1 expression is associated with poor prognosis (9). Recently, tumor mutational burden (TMB) has demonstrated prospects in clinical response. The objective response rate (ORR) and progression-free survival (PFS) in ICIs therapy are associated with high-TMB (TMB-H) (10). However, no significance association has been identified between TMB and overall prognosis (11). Neither PD-L1 nor TMB can specifically aid patient selection (12). Thus, it remains necessary to identify reliable biomarkers to improve ICIs therapy.

Immunotherapy targets the immune system or tumor microenvironment and activates the immune system against tumors by different immune cells (13). Lymphocytes that infiltrate the tumor (tumor-infiltrating lymphocytes, TILs) play an important role (13). Previous studies (14,15) have demonstrated that TILs density, particularly CD8⁺TILs and regulatory T cells (Tregs), may predict clinical outcomes for ICIs therapy. In most types of human cancer, consistent intra-tumoral infiltration of CD8⁺ TILs and helper T cells 1 (Th1) are associated with favorable prognosis and better survival outcomes (16). Pretreatment incidence of CD8⁺ T cells density at the invasive

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margin is associated with the PD-1/PD-L1 pathway and is an important determinant of improved outcomes in patients with melanoma (14). Apart from TILs density and distribution, their phenotypes also play key roles. A study demonstrated that high numbers of pre-existing memory T cells are potential biomarkers in patients with melanoma treated with CTLA-4 antibody (17). Another randomized trial in a hepatocellular carcinoma cohort that received preoperative nivolumab or ipilimumab treatment demonstrated that increased effector T cells were associated with complete response (CR) (18). The present study summarizes TILs phenotypes in tumor tissues, lymphocyte subtypes and serum cytokines in peripheral blood (PB). Their associations with clinical outcomes and probable prognostic biomarkers for patients with NSCLC in ICIs therapy are also summarized.

2. TILs in tumor tissues

TILs are a group of tumor invasive cells that exert antigenic effects, which were initially discovered in tumor tissue of mice in 1986 (19). PD-L1 is expressed on both TILs and tumor tissues (20). Recently, a model of tumor immune microenvironment based on TIL status and PD-L1 expression has been established (21). In this model, tumor microenvironments were classified into four types (21), including type I (PD-L1 positive with TILs positive), type II (PD-L1 negative with TILs negative), type III (PD-L1 positive with TILs negative) and type IV (PD-L1 negative with TILs positive). The type I tumor microenvironment has favorable response to PD-1/PD-L1 checkpoint inhibitors therapy (21). Furthermore, TILs phenotypes in tumor tissues play a crucial role in ICIs therapy (22) (Table 1).

CD4 and CD8 TILs. The activation of T cells involves antigen delivery from the major histocompatibility complex (MHC) molecules on antigen-presenting cells to the corresponding T cell receptor (TCR) on naïve T cells (23). MHC I molecules combine with CD8⁺ T cells and MHC II molecules bind to CD4⁺ T cells to assist antigen recognition (23). CD8⁺ T cells are the primary cytotoxic cells and critical for cell-mediated antitumor immune responses (23). CD4⁺ T cells are predominantly expressed on helper T cells (Th), which regulate or help other lymphocytes (23) (Fig. 1).

To study the role of TILs, Tokito *et al* (24) and El-Guindy *et al* (25) assessed the combination of PD-L1 expression and CD8 TIL density via immunohistochemical analysis. Both studies reported that PD-L1⁺/CD8^{LOW} patients had a short overall survival (OS) time, while PD-L1⁺/CD8^{HIGH} patients had a long OS time (24,25). Kim *et al* (26) demonstrated that low PD-L1 expression and high CD8⁺ TILs levels are associated with a favorable prognosis in resectable NSCLC. However, another study assessing PD-L1 expression, TIL status and their combination in NSCLC resectable patients treated by chemotherapy or targeted therapy, reported that neither was an independent prognostic factor of survival (27).

In tumors with DNA mismatch repair deficiencies, density of CD8⁺ TILs and PD-L1 expression, and survival rates are higher (28). This suggests that patients with DNA mismatch repair deficiencies may benefit from PD-1 therapy (28). A study involving 797 patients with NSCLC demonstrated that stromal

CD8⁺ TIL density had independent prognostic effect on each pStage and was a probable biomarker for TNM stage (29). Immune states were evolutive and specimens revealed immune cell invasion at the time point. In addition, a higher number of CD8⁺ T and/or CD4⁺ T cells in tumor stroma of resected or biopsy specimens were independent advantageous prognostic factors for NSCLC (15,30,31). A meta-analysis involving 8,600 patients with NSCLC from 29 studies demonstrated that CD8⁺ TILs were associated with improved OS (15). Nevertheless, CD4⁺ T cells were only associated with OS when assessed in tumor stroma compared with the tumor cell nests (15). An analysis of 129 surgically resected pathological specimens from patients with lung carcinoma with stage II/III confirmed higher levels of CD8⁺ cells, CD45RO⁺ memory T cells and CD57⁺ effector T cell in the peritumoral stroma were associated with a longer OS time (32). Taken together, these results suggest that CD8⁺ T cells mediate stronger anti-tumor responses compared with CD4.

In patients with NSCLC who receive nivolumab therapy, low PD-1 expression on CD8⁺ T cells is a favorable prognostic factor, and CD8⁺ TILs in tumor tissues exerts a predictive role (33). However, in a phase II trial of NSCLC patients treated with pembrolizumab, invasion of CD8⁺ T cells was not associated with PFS from the beginning of local ablative therapy (34). In 33 recurrent advanced patients with NSCLC, treated with nivolumab, no statistical significance was observed between CD8⁺ T cells and clinical outcomes (35). Thus, all progressive disease (PD) patients could not continue nivolumab treatment because of the increased number of stromal transforming growth factor- β (TGF β 1)-induced protein/low intra-tumoral CD8⁺ T cells (36). In lung adenocarcinoma, a study revealed that high CD8⁺ TILs density is associated with poor clinical outcomes, with regards to mortality and recurrence, particularly in non-smokers (36). The high PD-1⁺ TILs to CD8⁺ TILs ratio was also associated with a worse prognosis (37). Low ratio of PD-1:CD8 resulted in a longer OS and disease-free survival (DFS) in resected patients using nivolumab, which suggests that the absence of the PD-1 receptor is an independent prognostic factor (33). The predictive role of CD8⁺ T cells in tumor tissues remains unclear, particularly after activation of the immune system by ICIs.

Zeng *et al* (38) reported that a high concentration of CD4⁺ T lymphocytes in tumor-associated stroma were significantly associated with survival. The association between concentrated CD4⁺ T cells in the stroma (not in intra-tumoral) and disease mortality were also observed in the patients (38,39). Furthermore, Hiraoka *et al* (39) demonstrated that high levels of CD4⁺ T cells in the tumor stroma are associated with favorable clinical outcomes. Wakabayashi *et al* (40) reported that only the CD4⁺ T cells in tumor stroma (not CD8⁺ T cells in tumor cell nests) were associated with favorable prognosis in patients with NSCLC. Another study failed to identify an association between CD4⁺ lymphocyte infiltration and response or survival to pembrolizumab (41).

Tregs. Tregs are a subtype of CD4⁺ T cells whose primary function is maintaining immune tolerance and preventing the development of autoimmune conditions. Forkhead box protein P3 (FOXP3) is a specific transcription factor of Tregs (42). It is well-known that the expansion of Tregs in

Table I. Biomarkers for clinical outcomes by checkpoint inhibitors for non-small cell lung cancer.

Biomarker	Numbers of patients, n	Treatment	Results	(Refs.)
TILs				
CD8 ⁺	100	Nivolumab	Resected patients with high CD8 ⁺ T cells lacking PD-1 inhibitory receptor had a longer OS	(27)
	33-51	Pembrolizumab	No association was observed between CD8 ⁺ T and clinical outcomes	(28,29)
	100	Nivolumab	Low PD-1/CD8 resulted in a longer OS and DFS in resected patients	(32)
CD4 ⁺	38	Pembrolizumab	No association was observed between CD4 ⁺ infiltration and outcomes	(36)
Treg	31	Pembrolizumab/Nivolumab	Patients with a high proportion of PD-L1 ^{high} Tregs had a favorable response	(39)
Blood T cells				
PD1 ⁺ CD4 ⁺	22	Pembrolizumab/ Nivolumab or Atezolizumab	A high percentage of PD1 ⁺ CD4 ⁺ was associated with a longer PFS	(46)
PD1 ⁺ CD8 ⁺	31	Nivolumab	Prolonged survival outcomes were observed in patients who had higher PD1 ⁺ CD8 ⁺ cells in baseline	(50)
CD4 ⁺ Tcm	18	Pembrolizumab/Nivolumab	Percentage of CD4 ⁺ Tcm cells was higher in SD and PR patients compared with PD patients	(54)
Tcm/Tec	22	Nivolumab	High CD4 ⁺ and CD8 ⁺ Tcm/Tec ratios were associated with extended PFS	(55)
Exhausted T	74	Nivolumab	Higher frequency of exhausted CD8 ⁺ T cells in the PB resulted in a longer OS time	(45)
Cytokines				
IFN- γ	17-287	Pembrolizumab/Nivolumab or Atezolizumab	Patients who had higher expression of IFN- γ gene had better treatment response and improved OS	(73-75)
TNF- α	26	Pembrolizumab/Nivolumab	Increased TNF- α levels resulted in better response and a longer survival time	(76)
Others				
Tim-3	18	Pembrolizumab/Nivolumab	Expression of Tim-3 in CD4 ⁺ and CD8 ⁺ T increased in PD patients and decreased in the SD and PR groups	(54)
Ki67	60	Nivolumab	Positive Ki67 rates decreased in patients with non-responding tumors	(58)
	79	Pembrolizumab/Nivolumab	Ki-67 _{D7/D0} >2.8 predicted better PFS and OS in both the tested and validated cohorts	(59)
TCR	40	Anti-PD-1/Anti-PD-L1	Patients with increased PD-1 ⁺ CD8 ⁺ TCR clonality following ICB treatment had a longer PFS	(67)

TILs, tumor-infiltrating lymphocytes; CD, cluster of differentiation; Treg, regulatory T cells; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; PD, progressive disease; SD, stable disease; PR, partial response; PD-L1, programmed cell death-ligand 1; Tcm, central memory T cells; Tec, effector T; Tem, effector memory T cells; IFN- γ , interferon- γ ; TNF- α , tumor necrosis factor α ; PB, peripheral blood; Tim-3, T cell immunoglobulin and mucin-domain containing-3; TCR, T cell receptor; ICB, immune checkpoint blocked.

lung cancer is associated with a poor outcome, whereby high levels of Tregs in stage I NSCLC tumors increase the risk of recurrence (43). A meta-analysis revealed that a high concentration of FOXP3 regulator T cells in tumor stroma was a negative biomarker, which was associated with shorter PFS (15). Increased infiltration of Tregs into core tumor regions has a negative effect on survival and is a poor independent OS factor in NSCLC (44). This effect may regulate the increased vicinity of CD8⁺ T cells.

In PD-1/PD-L1 blockade immunotherapy, the density of PD-L1⁺CD4⁺ CD25⁺ Tregs in tumor stroma may provide a basis for diagnosis (45). Patients with a high proportion of PD-L1^{high}CD25⁺CD4⁺ TILs have a favorable response (45). However, some studies have failed to identify an association between the density of FOXP3 and the prognosis of patients in operable NSCLC (40,46,47). A study including 80 patients with NSCLC demonstrated that improved OS is associated with an increased number of FOXP3⁺ Tregs in tumor stroma.

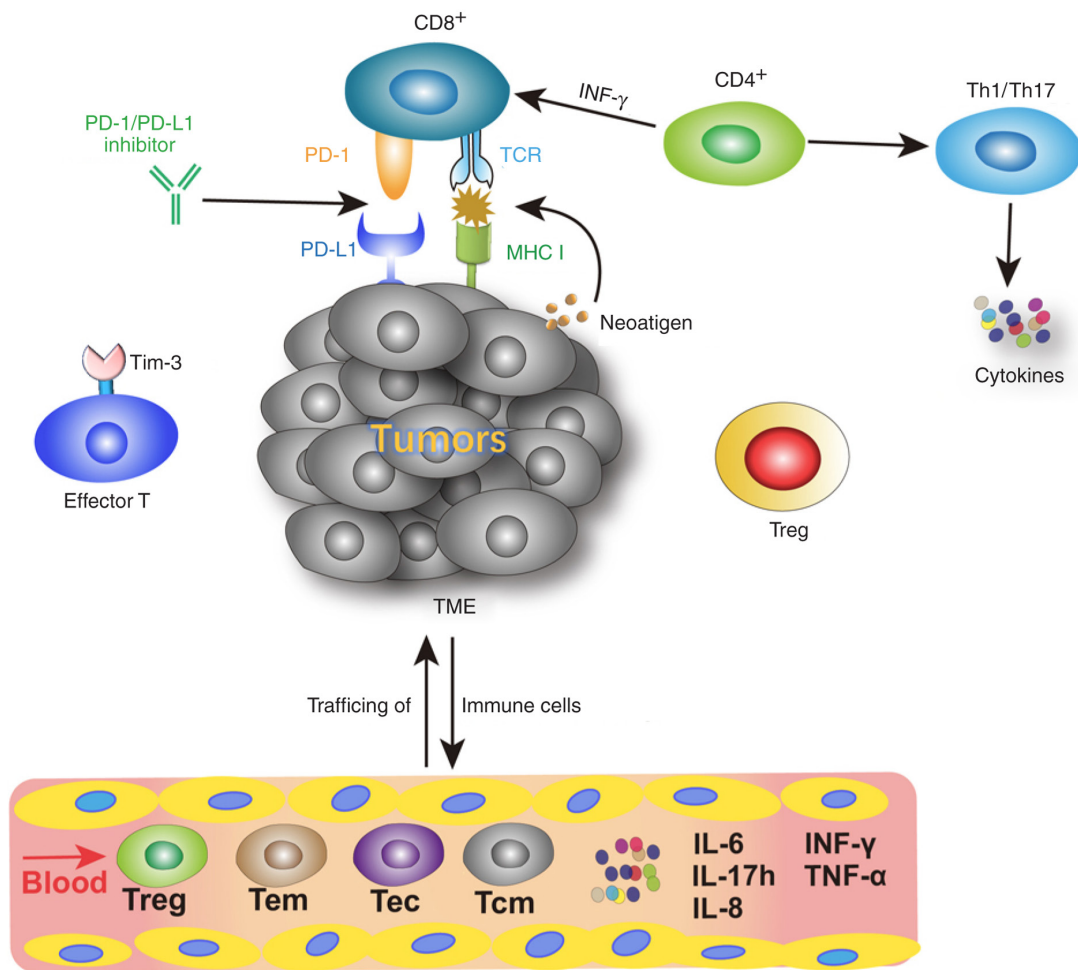


Figure 1. Representation of main biomarkers that predict the efficacy of PD-1/PD-L1 inhibitors therapy. PD-1/PD-L1 inhibitors targeted the PD-L1 status. Tumor infiltrating lymphocytes, such as CD8⁺, Th1/Th17 and Treg, reflect immune surveillance, which can be reactivated by agents. Peripheral T lymphocyte subtypes and cytokines exert an antitumor response and effect the immune status. PD-L1, programmed death-ligand 1; CD, cluster of differentiation; TME, tumor microenvironment; Tem, effector memory T; Tec, effector T; Tcm, central memory T; TCR, T cell receptor; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor α; Tim-3, T cell immunoglobulin and mucin-domain containing-3; Th, helper T cells; MHC, the major histocompatibility complex; Treg, regulatory T cells; IL, interleukin.

In addition, elevated density of intra-tumoral CD8⁺ cells and FOXP3 cells were independently associated with positive OS (48). The index of CD8^{low}/FOXP3^{high} is associated with poor OS and DFS, which is also an independent prognostic factor for DFS (49).

Blood biomarkers. Blood-based biomarkers are easily accessible during therapy, as disruption via intra- and inter-tumor heterogeneity is prevented (50). Some vital peripheral lymphocyte subgroups associated with responses to immunotherapy are presented in Fig. 1. Previous studies of circulating biomarkers for ICIs therapy include different T cells subtypes and cytokines (Table I).

3. Peripheral T lymphocytes

In advanced lung cancer patients, PB CD3⁺, CD4⁺ and natural killer (NK) cell counts are significantly lower compared with healthy individuals (51) and the effects of active CD8⁺ cells are seriously damaged (52). Proliferation of CD8⁺ T cells in PB have been assessed as biomarkers. Decreased

levels of CD4⁺ and CD8⁺ T cells are associated with disease progression (53). A recent study in the IRCCS Hospital San Martino in Italy suggested that pre-treatment distribution of T cells subpopulation and PD-1 expression on T lymphocytes assisted in predicting PD-1 blocked efficacy for patients with NSCLC (54). Patients with higher percentages of total CD3⁺, CD4⁺ and CD8⁺ T cells prior to treatment were associated with better PFS and OS (54). Inomata *et al* (55) demonstrated that patients treated with ICIs, with high frequencies of PD1⁺CD4⁺ T cells, had longer PFS than patients with low percentages. However, no significant associations were observed between PFS and frequencies of PD1⁺CD8⁺ TILs or cytokines levels in plasma. Previous studies have reported that the percentage of PD1⁺CD8⁺ T lymphocytes is associated with poor clinical outcomes in some tumor patients, in the absence of ICIs therapy (56-58). A study on immune cells in the PB of patients with NSCLC who received nivolumab demonstrated the significance of NK cells and PD1⁺CD8⁺ cells (59). Prolonged survival outcomes were observed in patients who had higher circulating NK cells and PD1⁺CD8⁺ cells in baseline (59). Conversely, decrease of circulating Tregs in response to

atezolizumab therapy is associated with favorable outcomes in patients with NSCLC (60).

Memory and effector T cells. Most T cells extend to differentiate into short-term living effector T cells (T_{ec}), which disappear quickly in the contraction phase (61). Antigen-specific memory T cells maintain high frequencies and can respond to antigenic stimulation with rapid effector function and proliferation (61). There are two main subsets of memory cells, effector memory T cells (T_{em}) and central memory T cells (T_{cm}) (61). Effector and memory T cells take up residency and become resident memory T cells (T_{rm}), which inhabit peripheral tissues (61). Memory T cells infiltration in lymph node metastases is an independent positive prognostic factors in patients with 1NSCLC (62). Higher levels of memory CD4⁺T, memory CD8⁺T and less naïve CD4⁺ T cells have been in observed in responders compared with non-responders in lung metastases, treated with stereotactic body radiotherapy (63). Julia *et al* (53) demonstrated that in patients with NSCLC treated with either 6 cycles of nivolumab or 4 cycles of pembrolizumab, the percentage of CD4⁺T_{cm} (CD4⁺CD45RO⁺CCR7⁺) prior to treatment is higher in stable disease (SD) and partial response (PR) patients compared with progressive disease (PD) patients, while a decrease in naïve CD4⁺ T cells was observed in SD and PR patients. The PD cohort was defined as patients whose sum of the maximum diameters of targeted lesions increased at least 20%, or patients displaying new metastasis (53). In a cohort of non-squamous NSCLC patients (n=22) treated with nivolumab, high CD4⁺ and CD8⁺ T_{cm}/T_{ec} ratios were associated with inflamed tumors and extended PFS (64). A tendency towards a higher percentage of CD45RA⁺CCR7⁺CD8⁺ T cells was detected in PR groups compared with PD groups at baseline and several time points during treatment with nivolumab (65). Higher percentages of CD95⁺CD60⁺CD8⁺ T cells were also observed (65). It was speculated that the local antigen encounter may have induced T cell differentiation and tissue egression of CD8⁺ T cells in PR patients (65). CD8⁺CD103⁺ serves as a marker of T_{rm} (66). A study demonstrated that high CD103⁺ TILs density is associated with the survival of patients with NSCLC at an early stage, and CD103⁺ may be a potential biomarker of tumor reactive CD8⁺ TIL (66). In advanced NSCLC patients (n=74) treated with nivolumab, the frequency of exhausted CD8⁺ T cells (CD8⁺PD1⁺Eomes⁺) prior to treatment notably decreases in patients whose disease progress is well controlled (54). In addition, a higher frequency of exhausted CD8⁺ T cells in PB is associated with a longer OS time (54).

4. Ki67

The nuclear protein, Ki67, functions in assessing cell proliferation and measuring the proliferative capacity of cells (67). Although Ki67 expression may not be visible at all active stages of cell division, the principles of irreparable DNA repair or the quiescent step are not yet available (67). In NSCLC patients treated with ICIs, the highest fold change of Ki-67 proliferation occurred among CD8⁺T cells (66). Thus, the activity of cycling CD8⁺ T cells increased in 70% of patients following ICIs therapy, which may be associated with clinical outcomes (66). A study aiming to monitor occupancy

of PD-1 on T cells with nivolumab demonstrated that Ki67 positivity rates of both nivolumab-binding and total T cells decreased in patients with non-responding tumors (68). Ki67 significantly decreases at the time of disease progression (68). In a study by Kim *et al* (69), the proliferative percentage of Ki67 among cycle PD-1⁺CD8⁺ T cells was assessed prior to treatment (D0) and 7 days after patients received the first dose of pembrolizumab or nivolumab (D7). The higher fold-change (Ki-67_{D7/D0}) revealed prolonged survival. Ki-67_{D7/D0}>2.8 predicted better PFS and OS, both in the independent tested and validated cohorts (69). Furthermore, some studies have demonstrated that ICIs treatment efficacy is associated with frequencies of PD1⁺Ki67⁺CD8⁺ T-lymphocytes or the ratio of PD1⁺Ki67⁺CD8⁺ T lymphocytes to tumor burden (70,71).

5. Tim-3

T cell immunoglobulin and mucin-domain containing-3 (Tim-3) is a co-inhibitory receptor expressed on IFN- γ -producing T cells, FOXP3⁺ Treg cells and innate immune cells (72). It functions in inhibiting Th1 responses and the expression of cytokines (72). Tim-3 regulates T cell exhaustion in TILs (72). Early accumulation of lymphocytes with high Tim-3 expression is associated with both primary and secondary resistance (73). Koyama *et al* (74) demonstrated that upregulation of Tim-3 was an indication of anti-PD1 treatment resistance in both the animal model and pleural effusion of patients with NSCLC. High Tim-3 levels expressed on CD8⁺ T cells are associated with poor tumor progression (75). In patients with lung adenocarcinoma who undergo surgery, positive Tim-3 expression is associated with positive PD-1 expression and high CD8⁺ TIL density (76). In addition, positive Tim-3 expression is associated with poor recurrence-free survival (RFS) and OS (76). In the study by Julia *et al* (53), Tim-3 expression in CD4⁺ and CD8⁺ T cells increased in PD patients, while the levels decreased in the SD and PR groups following anti-PD1 therapy (53). Taken together, these results suggest that Tim-3 may be an underlying negative biomarker of disease progression following immunotherapy.

6. TCR

TCR specifically targets antigenic peptides on the cell surface of human leukocyte antigen class I/ β -2-microglobulin complexes (77). TCR and its signaling molecules accumulate in T cells or tumor cells, resulting in the formation and transmission cascade of immune synapse (IS), and perform cytotoxic T lymphocyte (CTL) effector functions (77). Activation of the host immune response against cancer cells includes recognition of neoantigen peptides by clonally proliferating TCRs. The highly variable complementarity determining region 3 (CDR3) determines the specificity and diversity of TCR (77). Recently, TCR-based biomarkers have been investigated and some predictive responses have been demonstrated as follows. In patients with melanoma who are treated with ipilimumab, the clinical outcome is positive in relation to a higher pretreatment abundance of TCR repertoire richness (78). The level of CDR3 diversity in patients with lung cancer is inferior compared with healthy individuals (79). Patients with a more advanced disease state or poorer immune

status have substantially lower CDR3 diversity (79). Peripheral PD1⁺CD8⁺ TCR diversity may predict clinical benefits of PD-1/PD-L1 blockade therapy, which was calculated based on the Shannon-Wiener index (80) by sequencing CDR3 semi quantitatively with multiplex PCR (81). A high diversity of TCR provides more opportunities for tumor neoantigen recognition and improves immune response. Patients with high diversity of TCR have a significantly longer PFS than those with low diversity (79,81).

7. Cytokines

Cytokines are small molecular proteins with extensive biological activity secreted by immune cells, which are associated with physical activity, toxicity of drugs, resistance of treatment and infection (82). In a lung cancer mouse model induced by K-Ras oncogene, IL-6 was demonstrated to suppress initiation of lung carcinoma by activating cytotoxic CD8⁺ T cells; however, it improved tumor growth by inducing cell proliferation (83). These findings were consistent with the negative prognostic role of IL-6 in patients with NSCLC (84). In addition, the expression levels of IL-17A and IL-8 are significantly associated with disease progression (85,86). However, inflammatory cytokines are associated with cachexia, pain, toxicity of drugs, resistance to treatment and physical activity, which are all prognostic factors of clinical outcomes and may be influenced by these factors (87). Excluding granule exocytosis and Fas ligand-mediated apoptosis induction, CTL also releases interferon- γ (IFN- γ) and tumor necrosis factor α (TNF- α) to induce cytotoxicity in tumor cells (88). Thus, these two cytokines may be important in predicting efficacy. Another study demonstrated that four IFN- γ signatures from genetic studies at baseline tumor biopsies were predictive biomarkers for longer OS and PFS following treatment with durvalumab (89). In baseline NSCLC tumors, higher IFN- γ signature thresholds are associated with higher ORR, longer median OS and median PFS (89). In an open-label, phase II, randomized controlled trial, the efficacy of atezolizumab vs. docetaxel in 287 patients with NSCLC was assessed. The results demonstrated that tumor patients with a higher expression of T-effector/IFN- γ gene signature had a longer OS in the atezolizumab treatment group (90). Karachaliou *et al* (91) reported the association between high IFN- γ gene expression in baseline and PFS following treatment with anti-PD1, as well as the tendency between high IFN- γ gene expression and OS in patients with NSCLC. In patients treated with ICIs, such as pembrolizumab, nivolumab and atezolizumab, disease progression and interstitial pneumonitis were observed if the IFN- γ levels in the blood were <10 IU/ml (92). Levels of IFN- γ in the PB of advanced or metastatic patients with NSCLC were associated with better treatment response at 3 months following anti-PD1 therapy. In addition, statistical analysis demonstrated that different levels of IFN- γ resulted in different PD-L1 expression levels (93).

Along with other proinflammatory factors, TNF- α recruits neutrophils, macrophages and lymphocytes to the place of damage and infection and subsequently activates these cells (82). TNF- α levels in the plasma are positively associated with the expression levels of PD-L1 and PD-L2 in total T cells, Th cells and CTL (94). Excluding IFN- γ and ILs, Boutsikou *et al* (93)

demonstrated that increased TNF- α levels in the PB result in better response and longer survival time following anti-PD1 therapy.

8. Conclusion

Although high PD-L1 expression and TMB-H are currently recognized as beneficial signals in the application of ICIs therapy in patients with lung cancer, >50% of patients do not exhibit durable responses (95). Thus, it remains unclear if patients will benefit from ICIs therapy. The present review summarized different potential biomarkers. CD8⁺TILs are considered important cytotoxic immune cells, and the accumulation of Tregs is associated with immune suppression (23). Memory and effector T lymphocytes in the PB exert antitumor activity (61), and TCR diversity indicates the probability of neoantigen recognition (77). Furthermore, cytokines reflect immune levels and are closely associated with survival (82). Although these biomarkers are controversial and their specific molecular mechanism remain unclear, a promising tumor immune landscape has been established. Neither tissues or blood biomarkers alone can accurately predict disease progression and clinical outcomes. Prospective studies should investigate additional potential biomarkers both *in vitro* and *in vivo*. Future studies should also focus on the development and validation of multi-marker assays. Notably, clinical experiments with large sample sizes should be designed and their accuracy should be verified.

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Authors' contributions

XW conceived and designed the present review, performed the literature review, and prepared and drafted the initial manuscript. LG helped perform the literature review. WH contributed to the design, manuscript writing and editing. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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