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

XIAOYING WEI, LING GU and WEI HENG

Department of Medicine, Respiratory, Emergency and Intensive Care Medicine, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215000, P.R. China

Received June 27, 2020; Accepted November 20, 2020

DOI: 10.3892/ol.2020.12350

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.

Contents

Correspondence to: Dr Wei Heng, Department of Medicine, Respiratory, Emergency and Intensive Care Medicine, The First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou, Jiangsu 215000, P.R. China
E-mail: sdfyyhw@163.com

Key words: biomarkers, immunotherapy, lymphocytes, cytokines, non-small cell lung cancer

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 been 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 actives 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

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 I).

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 $PD\text{-}L1^+/CD8^{LOW}$ patients had a short overall survival (OS) time, while $PD\text{-}L1^-/CD8^{HIGH}$ 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 nets (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 antitumor 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 (TGFβ1)-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 $PD\text{-}1^+$ 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 Nivolumab	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 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.

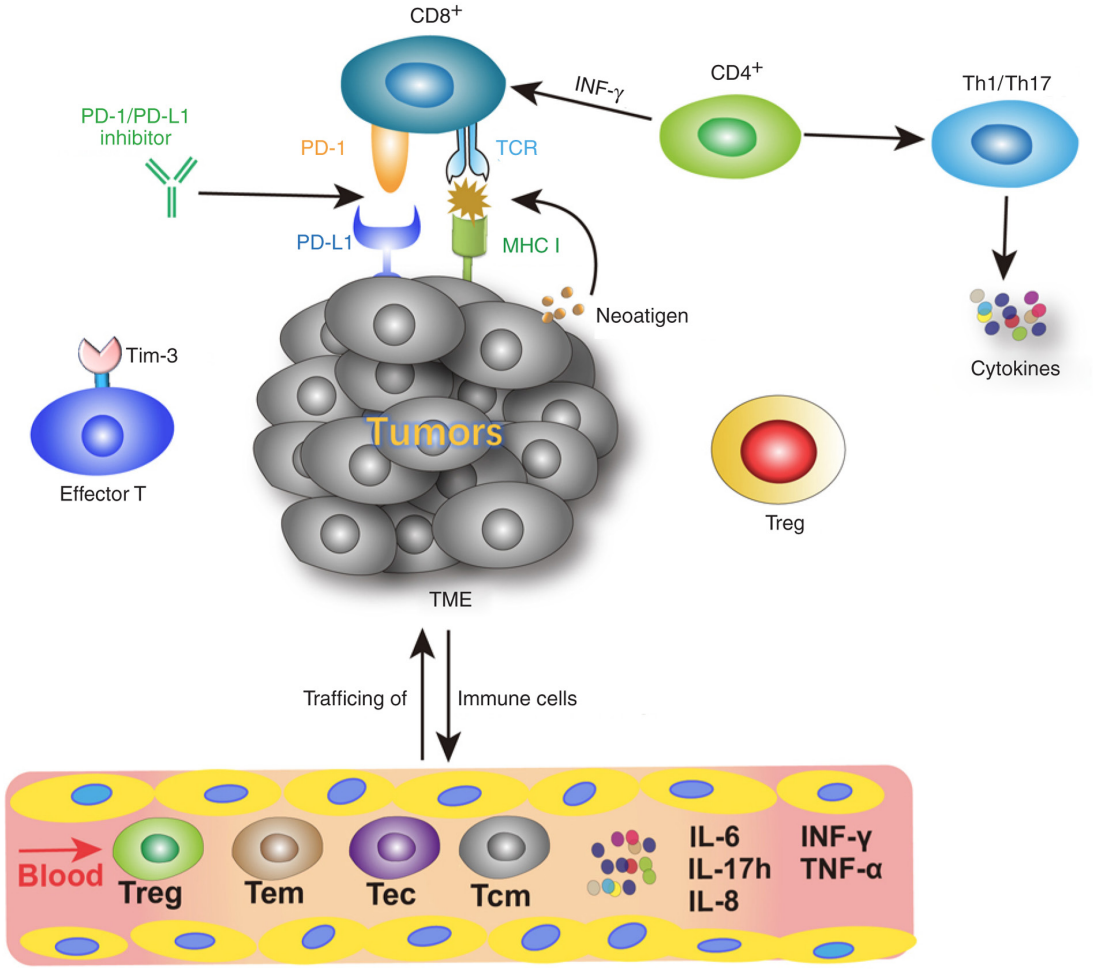

Figure 1. Representation of main biomarkers that predict the efficacy of PD-1/PD-L1 inhibitors therapy. PD-1/PD-L1 inhibitors targeted the PD-L1 status. Tumor infiltrating lymphocytes, such as $CD8^+$, Th1/Th17 and Treg, reflect immune surveillance, which can be reactivated by agents. Peripheral T lymphocyte 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 (Tec), 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 (Tem) and central memory T cells (Tcm) (61). Effector and memory T cells take up residency and become resident memory T cells (Trm), 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 $CD4^+$Tcm ($CD4^+CD45RO^+CCR7^+$) 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 $CD8^+$ Tcm/Tec 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 Trm (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 $PD\text{-}1^+CD8^+$ 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 ($Ki\text{-}67_{D7/D0}$) revealed prolonged survival. $Ki\text{-}67_{D7/D0}>2.8$ 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-r-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 actives 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.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

Not applicable.

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.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
3. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, *et al*: Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 373: 123-135, 2015.
4. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, *et al*: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373: 1627-1639, 2015.
5. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, *et al*: Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 387: 1540-1550, 2016.
6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, *et al*: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454, 2012.
7. Borghaei H BJ, Horn L, Ready N, Steins M, Felip E, Paz-Ares L, Xx X, Barlesi F, Antonia S and Fayette J: P2.35: nivolumab vs docetaxel in advanced NSCLC: CheckMate 017/057 2-Y update and exploratory cytokine profile analysis. Track: Immunotherapy J Thorac Oncol 11: S237-S238, 2016.
8. Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, Plimack ER, Vaena D, Grimm MO, Bracarda S, *et al*: Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): A multicentre, single-arm, phase 2 trial. Lancet Oncol 18: 312-322, 2017.
9. Zhang M, Li G, Wang Y, Wang Y, Zhao S, Haihong P, Zhao H and Wang Y: PD-L1 expression in lung cancer and its correlation with driver mutations: A meta-analysis. Sci Rep 7: 10255, 2017.
10. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, Stephens PJ, Daniels GA and Kurzrock R: Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 16: 2598-2608, 2017.
11. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, Felip E, van den Heuvel MM, Ciuleanu TE, Badin F, *et al*: First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 376: 2415-2426, 2017.
12. Yu Y, Zeng D, Ou Q, Liu S, Li A, Chen Y, Lin D, Gao Q, Zhou H, Liao W and Yao H: Association of survival and immune-related Biomarkers with immunotherapy in patients with non-small cell lung cancer. A meta-analysis and individual patient-level analysis. JAMA Netw Open 2: e196879, 2019.
13. Kitamura T, Qian BZ and Pollard JW: Immune cell promotion of metastasis. Nat Rev Immunol 15: 73-86, 2015.
14. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, *et al*: PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515: 568-571, 2014.
15. Geng Y, Shao Y, He W, Hu W, Xu Y, Chen J, Wu C and Jiang J: Prognostic role of tumor-infiltrating lymphocytes in lung cancer: A meta-analysis. Cell Physiol Biochem 37: 1560-1571, 2015.
16. Fridman WH, Pages F, Sautes-Fridman C and Galon J: The immune contexture in human tumours: Impact on clinical outcome. Nat Rev Cancer 12: 298-306, 2012.
17. Subrahmanyam PB, Dong Z, Gusenleitner D, Giobbie-Hurder A, Severgnini M, Zhou J, Manos M, Eastman LM, Maecker HT and Hodi FS: Distinct predictive biomarker candidates for response to anti-CTLA-4 and anti-PD-1 immunotherapy in melanoma patients. J Immunother Cancer 6: 18, 2018.
18. Kaseb AO, Vence L, Blando J, Yadav SS, Ikoma N, Pestana RC, Vauthey JN, Allison JP and Sharma P: Immunologic correlates of pathologic complete response to preoperative immunotherapy in hepatocellular carcinoma. Cancer Immunol Res 7: 1390-1395, 2019.
19. Rosenberg SA, Spiess P and Lafreniere R: A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 233: 1318-1321, 1986.
20. He Y, Rozeboom L, Rivard CJ, Ellison K, Dziadziuszko R, Yu H, Zhou C and Hirsch FR: PD-1, PD-L1 protein expression in non-small cell lung cancer and their relationship with tumor-infiltrating lymphocytes. Med Sci Monit 23: 1208-1216, 2017.
21. Teng MW, Ngiow SF, Ribas A and Smyth MJ: Classifying cancers based on T-cell Infiltration and PD-L1. Cancer Res 75: 2139-2145, 2015.
22. Park J, Kwon M, Kim KH, Kim TS, Hong SH, Kim CG, Kang SG, Moon JH, Kim EH, Park SH, *et al*: Immune checkpoint inhibitor-induced reinvigoration of tumor-infiltrating $CD8^+$ T cells is determined by their differentiation status in glioblastoma. Clin Cancer Res 25: 2549-2559, 2019.
23. Natarajan K, Jiang J, May NA, Mage MG, Boyd LF, McShan AC, Sgourakis NG, Bax A and Margulies DH: The role of molecular flexibility in antigen presentation and T cell receptor-mediated signaling. Front Immunol 9: 1657, 2018.
24. Tokito T, Azuma K, Kawahara A, Ishii H, Yamada K, Matsuo N, Kinoshita T, Mizukami N, Ono H, Kage M and Hoshino T: Predictive relevance of PD-L1 expression combined with CD8+ TIL density in stage III non-small cell lung cancer patients receiving concurrent chemoradiotherapy. Eur J Cancer 55: 7-14, 2016.
25. El-Guindy DM, Helal DS, Sabry NM and Abo El-Nasr M: Programmed cell death ligand-1 (PD-L1) expression combined with CD8 tumor infiltrating lymphocytes density in non-small cell lung cancer patients. J Egypt Natl Canc Inst 30: 125-131, 2018.
26. Kim SH, Go SI, Song DH, Park SW, Kim HR, Jang I, Kim JD, Lee JS and Lee GW: Prognostic impact of CD8 and programmed death-ligand 1 expression in patients with resectable non-small cell lung cancer. Br J Cancer 120: 547-554, 2019.
27. Lin G, Fan X, Zhu W, Huang C, Zhuang W, Xu H, Lin X, Hu D, Huang Y, Jiang K, *et al*: Prognostic significance of PD-L1 expression and tumor infiltrating lymphocyte in surgically resectable non-small cell lung cancer. Oncotarget 8: 83986-83994, 2017.
28. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, *et al*: PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 372: 2509-2520, 2015.
29. Donnem T, Hald SM, Paulsen EE, Richardsen E, Al-Saad S, Kilvaer TK, Brustugun OT, Helland A, Lund-Iversen M, Poehl M, *et al*: Stromal $CD8^+$ T-cell density-a promising supplement to tnm staging in non-small cell lung cancer. Clin Cancer Res 21: 2635-2643, 2015.
30. Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM and Busund LT: Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 14: 5220-5227, 2008.
31. Kawai O, Ishii G, Kubota K, Murata Y, Naito Y, Mizuno T, Aokage K, Saijo N, Nishiwaki Y, Gemma A, *et al*: Predominant infiltration of macrophages and $CD8^+$ T cells in cancer nests is a significant predictor of survival in stage IV non-small cell lung cancer. Cancer 113: 1387-1395, 2008.
32. Sepesi B, Cuentes EP, Canales JR, Behrens C, Correa A, Antonoff M, Gibbons DL, Heymach WH, Mehran R, Rice DC, *et al*: Tumor-infiltrating lymphocytes and overall survival in surgically resected tage II and III non-small cell lung cancer. Int J Rad Oncology Biol Phys 98: 223, 2017.
33. Mazzaschi G, Madeddu D, Falco A, Bocchialini G, Goldoni M, Sogni F, Armani G, Lagrasta CA, Lorusso B, Mangiaracina C, *et al*: Low PD-1 expression in cytotoxic $CD8^+$ tumor-infiltrating lymphocytes confers an immune-privileged tissue microenvironment in NSCLC with a prognostic and predictive value. Clin Cancer Res 24: 407-419, 2018.
34. Bauml JM, Mick R, Ciunci C, Aggarwal C, Davis C, Evans T, Deshpande C, Miller L, Patel P, Alley E, *et al*: Pembrolizumab after completion of locally ablative therapy for oligometastatic non-small cell lung cancer: A Phase 2 Trial. JAMA Oncol 5: 1283-1280, 2019.
35. Nakazawa N, Yokobori T, Kaira K, Turtoi A, Baatar S, Gombodorj N, Handa T, Tsukagoshi M, Ubukata Y, Kimura A, *et al*: High stromal TGFBI in lung cancer and intratumoral CD8-positive T cells were associated with poor prognosis and therapeutic resistance to immune checkpoint inhibitors. Ann Surg Oncol 27: 933-942, 2020.
36. Kinoshita T, Kudo-Saito C, Muramatsu R, Fujita T, Saito M, Nagumo H, Sakurai T, Noji S, Takahata E, Yaguchi T, *et al*: Determination of poor prognostic immune features of tumour microenvironment in non-smoking patients with lung adenocarcinoma. Eur J Cancer 86: 15-27, 2017.

37. Koh J, Go H, Keam B, Kim MY, Nam SJ, Kim TM, Lee SH, Min HS, Kim YT, Kim DW, *et al*: Clinicopathologic analysis of programmed cell death-1 and programmed cell death-ligand 1 and 2 expressions in pulmonary adenocarcinoma: Comparison with histology and driver oncogenic alteration status. Mod Pathol 28: 1154-1166, 2015.
38. Zeng DQ, Yu YF, Ou QY, Li XY, Zhong RZ, Xie CM and Hu QG: Prognostic and predictive value of tumor-infiltrating lymphocytes for clinical therapeutic research in patients with non-small cell lung cancer. Oncotarget 7: 13765-13781, 2016.
39. Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y, Itoh T, Ohbuchi T, Kondo S and Katoh H: Concurrent infiltration by $CD8^+$ T cells and $CD4^+$ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer 94: 275-280, 2006.
40. Wakabayashi O, Yamazaki K, Oizumi S, Hommura F, Kinoshita I, Ogura S, Dosaka-Akita H and Nishimura M: $CD4^+$ T cells in cancer stroma, not $CD8^+$ T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. Cancer Sci 94: 1003-1009, 2003.
41. Hu-Lieskovan S, Lisberg A, Zaretsky JM, Grogan TR, Rizvi H, Wells DK, Carroll J, Cummings A, Madrigal J, Jones B, *et al*: Tumor characteristics associated with benefit from pembrolizumab in advanced non-small cell lung cancer. Clin Cancer Res 25: 5061-5068, 2019.
42. Nishikawa H and Sakaguchi S: Regulatory T cells in cancer immunotherapy. Curr Opin Immunol 27: 1-7, 2014.
43. Petersen RP, Campa MJ, Sperlazza J, Conlon D, Joshi MB, Harpole DH Jr and Patz EF Jr: Tumor infiltrating $Foxp3^+$ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients. Cancer 107: 2866-2872, 2006.
44. Barua S, Fang P, Sharma A, Fujimoto J, Wistuba I, Rao AUK and Lin SH: Spatial interaction of tumor cells and regulatory T cells correlates with survival in non-small cell lung cancer. Lung Cancer 117: 73-79, 2018.
45. Wu SP, Liao RQ, Tu HY, Wang WJ, Dong ZY, Huang SM, Guo WB, Gou LY, Sun HW, Zhang Q, *et al*: Stromal PD-L1-positive regulatory T cells and PD-1-positive CD8-positive T cells define the response of different subsets of non-small cell lung cancer to PD-1/PD-L1 blockade immunotherapy. J Thorac Oncol 13: 521-532, 2018.
46. Giatromanolaki A, Banham AH, Harris AL and Koukourakis MI: FOXP3 infiltrating lymphocyte density and PD-L1 expression in operable non-small cell lung carcinoma. Exp Lung Res 45: 76-83, 2019.
47. Sakai K, Maeda S, Yamada Y, Chambers JK, Uchida K, Nakayama H, Yonezawa T and Matsuki N: Association of tumour-infiltrating regulatory T cells with adverse outcomes in dogs with malignant tumours. Vet Comp Oncol 16: 330-336, 2018.
48. Jackute J, Zemaitis M, Pranys D, Sitkauskiene B, Miliauskas S, Bajoriunas V, Lavinskiene S and Sakalauskas R: The prognostic influence of tumor infiltrating $Foxp3^+CD4^+$, $CD4^+$ and $CD8^+$ T cells in resected non-small cell lung cancer. J Inflamm (Lond) 12: 63, 2015.
49. Kinoshita F, Takada K, Yamada Y, Oku Y, Kosai K, Ono Y, Tanaka K, Wakasu S, Oba T, Osoegawa A, *et al*: Combined Evaluation of tumor-nfiltrating CD8 + and FoxP3 + lymphocytes provides accurate prognosis in stage IA lung adenocarcinoma. Ann Surg Oncol 27: 2102-2109, 2019.
50. De Mattos-Arruda L, Cortes J, Santarpia L, Vivancos A, Tabernero J, Reis-Filho JS and Seoane J: Circulating tumour cells and cell-free DNA as tools for managing breast cancer. Nat Rev Clin Oncol 10: 377-389, 2013.
51. Wang WJ, Tao Z, Gu W and Sun LH: Variation of blood T lymphocyte subgroups in patients with non- small cell lung cancer. Asian Pac J Cancer Prev 14: 4671-4673, 2013.
52. Xu L, Chen D, Lu C, Liu X, Wu G and Zhang Y: Advanced lung cancer is associated with decreased expression of perforin, CD95, CD38 by circulating CD3+CD8+ T lymphocytes. Ann Clin Lab Sci 45: 528-532, 2015.
53. Julia EP, Mando P, Rizzo MM, Cueto GR, Tsou F, Luca R, Pupareli C, Bravo AI, Astorino W, Mordoh J, *et al*: Peripheral changes in immune cell populations and soluble mediators after anti-PD-1 therapy in non-small cell lung cancer and renal cell carcinoma patients. Cancer Immunol Immunother 68: 1585-1596, 2019.
54. Ottonello S, Genova C, Cossu I, Fontana V, Rijavec E, Rossi G, Biello F, Dal Bello MG, Tagliamento M, Alama A, *et al*: Association between response to nivolumab treatment and peripheral blood lymphocyte subsets in patients with non-small cell lung cancer. Front Immunol 11: 125, 2020.
55. Inomata M, Kado T, Okazawa S, Imanishi S, Taka C, Kambara K, Hirai T, Tanaka H, Tokui K, Hayashi K, *et al*: Peripheral PD1-positive CD4 T-lymphocyte count can predict progression-free survival in patients with non-small cell lung cancer receiving immune checkpoint inhibitor. Anticancer Res 39: 6887-6893, 2019.
56. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC and Kwon ED: PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res 13: 1757-1761, 2007.
57. Muenst S, Soysal SD, Gao F, Obermann EC, Oertli D and Gillanders WE: The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 139: 667-676, 2013.
58. Hsu MC, Hsiao JR, Chang KC, Wu YH, Su IJ, Jin YT and Chang Y: Increase of programmed death-1-expressing intratumoral CD8 T cells predicts a poor prognosis for nasopharyngeal carcinoma. Mod Pathol 33: 1393-1403, 2010.
59. Mazzaschi G, Facchinetti F, Missale G, Canetti D, Madeddu D, Zecca A, Veneziani M, Gelsomino F, Goldoni M, Buti S, *et al*: The circulating pool of functionally competent NK and CD8+ cells predicts the outcome of anti-PD1 treatment in advanced NSCLC. Lung Cancer 127: 153-163, 2019.
60. Zhuo M, Chen H, Zhang T, Yang X, Zhong J, Wang Y, An T, Wu M, Wang Z, Huang J and Zhao J: The potential predictive value of circulating immune cell ratio and tumor marker in atezolizumab treated advanced non-small cell lung cancer patients. Cancer Biomark 22: 467-476, 2018.
61. Schenkel JM and Masopust D: Tissue-resident memory T cells. Immunity 41: 886-897, 2014.
62. Kilvaer TK, Paulsen EE, Khanehkenari MR, Al-Saad S, Johansen RM, Al-Shibli K, Bremnes RM, Busund LT and Donnem T: The presence of intraepithelial CD45RO+ cells in resected lymph nodes with metastases from NSCLC patients is an independent predictor of disease-specific survival. Br J Cancer 114: 1145-1151, 2016.
63. Liu C, Hu Q, Xu B, Hu X, Su H, Li Q, Zhang X, Yue J and Yu J: Peripheral memory and naïve T cells in non-small cell lung cancer patients with lung metastases undergoing stereotactic body radiotherapy: Predictors of early tumor response. Cancer Cell Int 19: 121, 2019.
64. Manjarrez-Orduno N, Menard LC, Kansal S, Fischer P, Kakrecha B, Jiang C, Cunningham M, Greenawalt D, Patel V, Yang M, *et al*: Circulating T cell subpopulations correlate with immune responses at the tumor site and clinical response to PD1 inhibition in non-small cell lung cancer. Front Immunol 9: 1613, 2018.
65. Kunert A, Basak EA, Hurkmans DP, Balcioglu HE, Klaver Y, van Brakel M, Oostvogels AAM, Lamers CHJ, Bins S, Koolen SLW, *et al*: CD45RA+CCR7- CD8 T cells lacking co-stimulatory receptors demonstrate enhanced frequency in peripheral blood of NSCLC patients responding to nivolumab. J Immunother Cancer 7: 149, 2019.
66. Djenidi F, Adam J, Goubar A, Durgeau A, Meurice G, de Montpréville V, Validire P, Besse B and Mami-Chouaib F: $CD8^+CD103^+$ tumor-infiltrating lymphocytes are tumor-specific tissue-resident memory T cells and a prognostic factor for survival in lung cancer patients. J Immunol 194: 3475-3486, 2015.
67. Iatropoulos MJ and Williams GM: Proliferation markers. Exp Toxicol Pathol 48: 175-181, 1996.
68. Osa A, Uenami T, Koyama S, Fujimoto K, Okuzaki D, Takimoto T, Hirata H, Yano Y, Yokota S, Kinehara Y, *et al*: Clinical implications of monitoring nivolumab immunokinetics in non-small cell lung cancer patients. JCI Insight 3: e59125, 2018.
69. Kim KH, Cho J, Ku BM, Koh J, Sun JM, Lee SH, Ahn JS, Cheon J, Min YJ, Park SH, *et al*: The first-week proliferative response of peripheral blood $PD\text{-}1^+CD8^+$ T cells predicts the response to Anti-PD-1 therapy in solid tumors. Clin Cancer Res 25: 2144-2154, 2019.
70. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, Xu W, Harmon S, Giles JR, Wenz B, *et al*: T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature 545: 60-65, 2017.
71. Kamphorst AO, Pillai RN, Yang S, Nasti TH, Akondy RS, Wieland A, Sica GL, Yu K, Koenig L, Patel NT, *et al*: Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients. Proc Natl Acad Sci USA 114: 4993-4998, 2017.

72. Kuchroo VK, Umetsu DT, DeKruyff RH and Freeman GJ: The TIM gene family: Emerging roles in immunity and disease. Nat Rev Immunol 3: 454-462, 2003.
73. Limagne E, Richard C, Thibaudin M, Fumet JD, Truntzer C, Lagrange A, Favier L, Coudert B and Ghiringhelli F: Tim-3/galectin-9 pathway and mMDSC control primary and secondary resistances to PD-1 blockade in lung cancer patients. Oncoimmunology 8: e1564505, 2019.
74. Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, Gandhi L, Redig AJ, Rodig SJ, Asahina H, *et al*: Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun 7: 10501, 2016.
75. Japp AS, Kursunel MA, Meier S, Mälzer JN, Li X, Rahman NA, Jekabsons W, Krause H, Magheli A, Klopf C, *et al*: Dysfunction of PSA-specific $CD8^+$ T cells in prostate cancer patients correlates with CD38 and Tim-3 expression. Cancer Immunol Immunother 64: 1487-1494, 2015.
76. Su H, Xie H, Dai C, Ren Y, She Y, Xu L, Chen D, Xie D, Zhang L, Jiang G and Chen C: Characterization of TIM-3 expression and its prognostic value in patients with surgically resected lung adenocarcinoma. Lung Cancer 121: 18-24, 2018.
77. Kuhns MS, Davis MM and Garcia KC: Deconstructing the form and function of the TCR/CD3 complex. Immunity 24: 133-139, 2006.
78. Postow MA, Manuel M, Wong P, Yuan J, Dong Z, Liu C, Perez S, Tanneau I, Noel M, Courtier A, *et al*: Peripheral T cell receptor diversity is associated with clinical outcomes following ipilimumab treatment in metastatic melanoma. J Immunother Cancer 3: 23, 2015.
79. Liu YY, Yang QF, Yang JS, Cao RB, Liang JY, Liu YT, Zeng YL, Chen S, Xia XF, Zhang K and Liu L: Characteristics and prognostic significance of profiling the peripheral blood T-cell receptor repertoire in patients with advanced lung cancer. Int J Cancer 145: 1423-1431, 2019.
80. Ruggiero E, Nicolay JP, Fronza R, Arens A, Paruzynski A, Nowrouzi A, Ürenden G, Lulay C, Schneider S, Goerdt S, *et al*: High-resolution analysis of the human T-cell receptor repertoire. Nat Commun 6: 8081, 2015.
81. Han J, Duan J, Bai H, Wang Y, Wan R, Wang X, Chen S, Tian Y, Wang D, Fei K, *et al*: TCR repertoire diversity of peripheral $PD\text{-}1^+CD8^+$ T cells predicts clinical outcomes after immunotherapy in patients with non-small cell lung cancer. Cancer Immunol Res 8: 146-154, 2020.
82. Kaplansky G and Bongrand P: Cytokines and chemokines. Cell Mol Biol (Noisy-le-grand) 74: 569-574, 2001.
83. Qu Z, Sun F, Zhou J, Li L, Shapiro SD and Xiao G: Interleukin-6 prevents the initiation but enhances the progression of lung cancer. Cancer Res 75: 3209-3215, 2015.
84. Silva EM, Mariano VS, Pastrez PRA, Pinto MC, Castro AG, Syrjanen KJ and Longatto-Filho A: High systemic IL-6 is associated with worse prognosis in patients with non-small cell lung cancer. PLoS One 12: e0181125, 2017.
85. Pan B, Che D, Cao J, Shen J, Jin S, Zhou Y, Liu F, Gu K, Man Y, Shang L and Yu Y: Interleukin-17 levels correlate with poor prognosis and vascular endothelial growth factor concentration in the serum of patients with non-small cell lung cancer. Biomarkers 20: 232-239, 2015.
86. Sanmamed MF, Perez-Gracia JL, Schalper KA, Fusco JP, Gonzalez A, Rodriguez-Ruiz ME, Oñate C, Perez G, Alfaro C, Martín-Algarra S, *et al*: Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. Ann Oncol 28: 1988-1995, 2017.
87. Seruga B, Zhang HB, Bernstein LJ and Tannock IF: Cytokines and their relationship to the symptoms and outcome of cancer. Nat Rev Cancer 8: 887-899, 2008.
88. Thomas DA and Massagué J: TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 8: 369-380, 2005.
89. Higgs BW, Morehouse CA, Streicher K, Brohawn PZ, Pilataxi F, Gupta A and Ranade K: Interferon gamma messenger RNA Signature in tumor biopsies predicts outcomes in patients with non-small cell lung carcinoma or urothelial cancer treated with durvalumab. Clin Cancer Res 24: 3857-3866, 2018.
90. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, *et al*: Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. Lancet 387: 1837-1846, 2016.
91. Karachaliou N, Gonzalez-Cao M, Crespo G, Drozdowskyj A, Aldeguer E, Gimenez-Capitan A, Teixido C, Molina-Vila MA, Viteri S, De Los Llanos Gil M, *et al*: Interferon gamma, an important marker of response to immune checkpoint blockade in non-small cell lung cancer and melanoma patients. Ther Adv Med Oncol 10: 1758834017749748, 2018.
92. Hirashima T, Kanai T, Suzuki H, Yoshida H, Matsushita A, Kawasumi H, Samejima Y, Noda Y, Nasu S, Tanaka A, *et al*: The Levels of Interferon-gamma release as a biomarker for non-small-cell lung cancer patients receiving immune checkpoint inhibitors. Anticancer Res 39: 6231-6240, 2019.
93. Boutsikou E, Domvri K, Hardavella G, Tsiouda D, Zarogoulidis K and Kontakiotis T: Tumour necrosis factor, interferon-gamma and interleukins as predictive markers of antiprogrammed cell-death protein-1 treatment in advanced non-small cell lung cancer: A pragmatic approach in clinical practice. Ther Adv Med Oncol 10: 1758835918768238, 2018.
94. Arrieta O, Montes-Servin E, Hernandez-Martinez JM, Cardona AF, Casas-Ruiz E, Crispin JC, Motola D, Flores-Estrada D and Barrera L: Expression of PD-1/PD-L1 and PD-L2 in peripheral T-cells from non-small cell lung cancer patients. Oncotarget 8: 101994-102005, 2017.
95. Patel SP and Kurzrock R: PD-L1 Expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther 14: 847-856, 2015.