Narrative review of cytokines and cell surface markers in the tumor microenvironment of stage I lung cancer

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Abstract: The possibility to apply the knowledge of cell surface markers and cytokines in the tumor microenvironment of early lung cancer (LC) in clinical practice, is still a developing facet of oncological medicine, but may possibly be a very fertile, not wholly explored facet. Although early diagnosis of LC through the efforts of LC screening has served an important role in decreasing LC mortality, cancer recurrence is also an equally important clinical concern. Cell surface markers and cytokines in the tumor microenvironment of LC may serve a clinical prognostic purpose. This is specifically with regard to risk stratification and subsequent identification of patients that may most benefit from early postoperative adjuvant therapy, based on their risk of recurrence. These molecules could also potentially serve as therapeutic targets. However, the roles and effects of various cytokines and cell surface markers in the immune microenvironment (IME) of LC and other cancers, as well as the interplay between these molecules and infiltrating immune cells, have not been fully elaborated, and there remains work to be done in this respect. This article attempts to discuss some of these cell surface markers and cytokines that may, in the future, serve as prognosticators for the early LC, possibly in the forms of stratification scores and models.

Keywords: Cytokines; cell markers; lung cancer (LC); immune; microenvironment

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Introduction

This review will focus on cell surface markers and molecules, as well as cytokines, and the possible roles they play with regard to prognosis in early-stage lung cancer (LC) and tumorigenesis. I present the following article in accordance with the Narrative Review reporting checklist (available at https://amj.amegroups.com/article/view/10.21037/amj-20-144/rc).

Methods

A PubMed search was performed using the phrases: "lung cancer", "immune prognostic factors", "stage I", which provided 88 search results. We further limited the publication dates to the last 10 years: 2009-01-01–2019-12-31, which filtered the search results down to 74 (*Figure 1*). We further excluded articles whose primary focus was specifically stated to be advanced-stage LC, articles we were unable to get full access to, and articles that were not relevant to the topic at hand, i.e., articles not focused on cytokines or cell surface markers. More relevant articles were found within the reference lists of the previously mentioned relevant articles. In total we reviewed 93 articles, to come up with a robust summary of the current available knowledge on cell surface markers and cytokines in the immune microenvironment (IME) associated with stage I and early-stage LC.

Cell surface markers and molecules

This section will focus on the findings of recent research as they relate to the functions and prognostic value of cell surface markers and molecules. It will include discussions

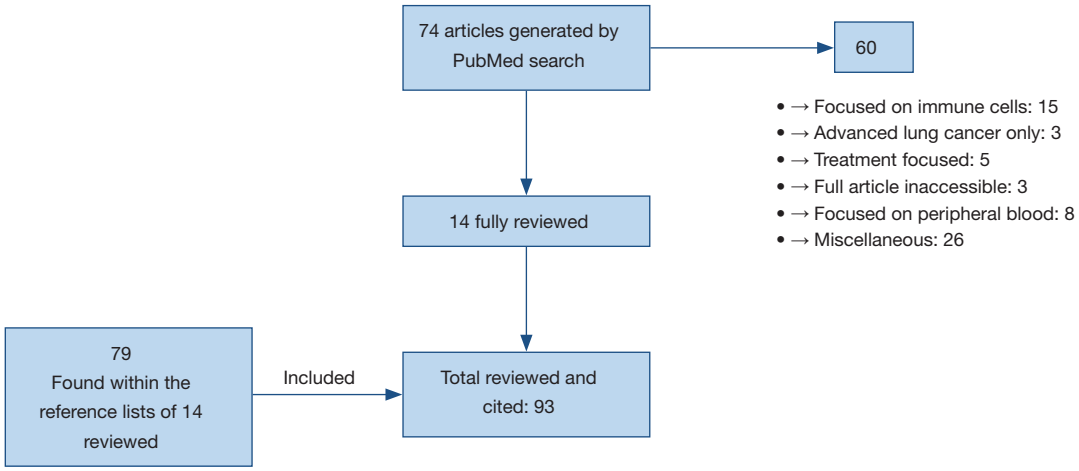

Figure 1 Flow diagram of the literature search process.

concerning the following molecules: 0X-40, PD-1/PD-L, CTLA-4 and HLA-II.

OX40

OX40, also known as CD134, is a member of the family of tumor necrosis factor (TNF) receptors. This receptor is highly expressed in various activated immune cells, including activated B-cells, T-cells, neutrophils, natural killer (NK) cells and macrophages. There are a number of mechanisms that have been suggested to explain the effects of OX40 in the tumor IME. Studies have shown that activation of OX40 leads to cytokine production that promotes CD4+ T-cell proliferation (1), Th17 T-cell proliferation via promoting the elaboration of interleukin (IL)-17 from T-cells activated by OX40 (2) and suppression of Foxp3 expression, a marker of regulatory T-cells (T-regs) (3). These findings lead to the notion that a high expression of OX40 would be associated with increased tumor inflammation and possibly an enhanced antitumor response, and is the basis for studies analyzing the effects of agonistic OX40 antibodies on tumors (4-7). Piconese *et al.* showed that agonistic OX40 monoclonal antibodies promoted cytotoxic T-cell proliferation, while inhibiting T-reg function via inactivation, thus having an overall antitumor effect in murine models (4). Weinberg *et al.* also showed in their murine study that stimulation of OX40 receptor by OX40 ligand (OX40-L) or agonist antibodies lead to T-cell proliferation and enhanced survival of effector T-cells, while also delaying the growth of tumors in mice that received OX40-L in a dose-dependent manner (7). Yokouchi showed in their LC murine models that administration of OX40-L alone lead to a significant reduction in tumor volume similar to radiotherapy (RDT) alone, compared with control models that received control IgG ($P<0.0001$); when both OX40-L and RDT were combined, this reduction in tumor volume was even more enhanced ($P<0.0001$) (8). An immunohistochemistry (IHC) study by Massarelli *et al.* on the prognostic significance of OX40 in the IME of surgically resected stage I–III non-small cell lung cancer (NSCLC) showed that high expression of OX40 in the IME was associated with an improved overall survival (OS) in univariate analysis [95% confidence interval (CI): 1.4–5.2, hazard ratio (HR): 2.68, $P=0.002$] and multivariate analysis ($P=0.004$), suggesting that OX40 appears to be an independent prognostic factor in the IME of LC. Although OX40 was shown to be positively correlated with improved survival, there did not appear to be any relationship between OX40 and clinicopathological factors in this study, including stage and histology. Furthermore, greater OX40 expression was also shown to be associated with increased expression of markers of immune activation, including the genes for interferon-gamma (IFN-γ), CD3, CD8 and others ($P\leq0.01$). This latter finding supports the notion that OX40 expression is associated with a robust immune response and a subsequent improved immune-mediated antitumor response (9). On the contrary, the expression of OX40 on tumor-infiltrating lymphocytes (TILs) in the tumor IME has been shown to be associated with a poorer prognosis in early-stage LC by He *et al.* (10). This study stained treatment-naïve resected stage I NSCLC tissue samples using IHC. Study results showed that a shorter disease-free survival (DFS) and OS were associated with

positive OX40 expression (OX40+) on TILs, compared with tumors with negative OX40 expression (OX40–), although these findings were statistically insignificant. Nonetheless, when OX40 expression was stratified by stage (stage IA OX40+ *vs.* OX40– *vs.* stage IB OX40+ *vs.* OX40–), statistically significant survival (P=0.0001) and recurrence (P=0.0001) disadvantages were disclosed to be associated with OX-40 expression, especially in stage IA disease. For example, TIL OX40 expression was compared between stage IA patients; OX40+ patients had 3.13 years median OS and 1.9 years median DFS, compared with OX40– stage IA patients, in whom median OS and DFS were 4.90 and 4.90 years, respectively (10). Although the study by He *et al.* showed a statistically significant negative correlation between DFS and TIL OX40 expression, the results may have been affected by the small sample size from which they were derived (n=139). Although there is little human-based LC-focused research on OX40 in the IME, the findings with regard to other human cancers that have shown OX40 in the IME of tumors to be a positive prognosticator for survival and lack of metastasis, provide further support that OX40 may probably be an indicator of positive prognosis in LC (11,12). Nevertheless, further research employing larger sample sizes is still required to ascertain whether OX40 expression, or stimulation of OX40 receptors truly serve as positive prognosticators that some preliminary studies have suggested, and if such prognostic effect is consistently applicable to LC.

Programmed death protein-1/programmed-death ligand (PD-1/PD-L)

PD-1, formerly known as B7-H1, is a cell surface protein whose functions include activity as an immune checkpoint. PD-1 is a transmembrane protein expressed on various immune cells including NK cells, B- and T-cells, activated monocytes and macrophages (13,14). PD-1 and its ligands (PD-L1, PD-L2) suppress the immune response; specifically, they promote T-reg activity via a number of suggested mechanisms, while suppressing non-T-reg CD4+ and CD8+ T-cells. It is thought that this suppressive action by PD-1 and its ligands are essential for preventing autoimmune disorders, by inhibiting the survival of self-reactive T-cells (15,16). While this immunosuppressive action of PD-1 and its ligands is essential for physiological immunity, the same activity infers negative prognosis in the setting of malignant tumors. This is supported by a number of studies that have shown an association between the expression of PD-1/PD-L1 in TILs and a poorer prognosis. He *et al.* showed a lower DFS rate in patients whose stage I NSCLC tumors expressed PD-L1 (PD-L1+), compared with those whose tumors did not express either PD-L1 or OX40. The DFS was significantly worse in patients whose tumors expressed both PD-L1 and OX40 (PD-L1+/OX40+) together, compared with patients who were both PD-L1 and OX40 negative (PD-L1–/OX40–), or only negative for one or the other (P=0.0001) (10). For example, at 3 years, the approximate DFS rate in stage IA PD-L1+/OX40+ tumors was 30%, 58% for PD-L1/OX40+ and 62% for PD-L1–/OX40– tumors; stage IB data also displayed a similar pattern and both were equally significant (P<0.0001). OS was also shown to negatively correlate with PD-L1 and/or OX40 expression. At 3 years, the approximate OS rate in stage IA patients with PD-L1+/OX40+ tumors was 58%. When either OX40 or PDL-1 was positive (PD-L1/OX40+) the approximate OS rate was 60%. When neither was positive (PD-L1–/OX40–) the OS rate was 72% (P<0.0001). A similar pattern was seen in stage IB tumors and was equally significant (P<0.0001) (10). It has been suggested that the overexpression of PD-L1 in LC, which has been shown to be a negative prognosticator, more consistently in the case of NSCLC, may be associated with the loss of BIN1, a tumor-suppressor protein responsible for downregulating PD-L1, amongst other immune-suppressive pathways (17). Targeted therapy has been developed against PD-1/PD-L, based on the premise of inhibiting the inhibitor of the immune response, thereby promoting the antitumoral effects of the immune system. Examples of such therapies include pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and cemiplimab (18-20). The negative prognostic effects of TILs expressing PD-1/PD-L1 in NSCLC may be associated with the increased glycolytic metabolism, as ascertained by FDG-PET, associated with tumors that express these immune checkpoint proteins (13,21). Such increased tumor metabolism may be responsible for promoting tumor survival. Another retrospective analysis of 113 stage I NSCLC samples also showed corroborating results, in which a low expression of PD-L1 in both tumors and tumor-infiltrating macrophages (TIMs) corresponded with a 95% 5-year OS, compared with a 20% 5-year OS when PD-L1 expression was high in both tumors and TIMs (P<0.001). Furthermore, in the intermediate group composed of high PD-L1 expression in either tumor cells (TCs) or TIMs (but not both), prognosis was less favorable (76% 5-year OS) than the low-expression group (in both tumors and TIMs), but better than the high-expression group (in both tumors

and TIMs) (P<0.001) (22). Another study assessing the mRNA levels of PD-L1 in NSCLC using data derived from The Cancer Genome Atlas (TCGA), rather than by IHC analysis like the previously aforementioned studies, showed that in patients with adenocarcinoma (ADC), PD-L1 levels were higher in recently-quit smokers (≤15 years) and current smokers, compared with smokers with a more remote smoking history (>15 years) and never-smokers (P<0.05). These findings support the notion that smoking potentially contributes to diminished immune function and pro-tumoral response (23). Another study by Rashed *et al.* showed PD-L1 to be negatively correlated with CD8+ T-cell expression, which further supports the notion that PD-L1 expression probably mediates suppression of cell-mediated antitumor response, at least in the case of NSCLC. In tumors with low CD8+ infiltration, 75% were PD-L1+, compared with only 25% PD-L1– (P=0.0045); conversely, only 31.3% of high CD8+ infiltrated tumors were PD-L1+, compared with 68.8% of these tumors, which were PD-L1– (P=0.004) (24). Similar findings that PD-L1 is associated with worse prognosis have been reproduced by a number of metanalyses (25,26). Paulsen *et al.* showed in their NSCLC study that high PD-1/PD-L1 expression within the tumor and in the surrounding stroma was associated with an improved disease-specific survival (DSS), in contrast with the aforementioned studies. High stromal PD-L1 expression was associated with a 5-year DSS rate of 67% (median: 235 months), compared with low stromal PD-L1 expression with a 53% 5-year DSS rate (median: 73 months; P=0.004). When the group was stratified according to histological subtype, the improved DSS associated with high PD-L1 expression was maintained in the squamous cell carcinoma (SCC group) (P=0.002) but lost in the ADC group (P=0.501). There was also a similar but insignificant prognostic advantage associated with high tumoral PD-L1 expression (63% 5-year DSS rate, 190 months median DSS), compared with low tumoral PD-L1 expression (56% 5-year DSS rate, 104 months median DSS; P=0.313). The study also showed a significant 5-year survival advantage with high *vs.* low stromal PD-L1 expression [59% (median: 87 months) *vs.* 47% (median: 44 months), respectively; P=0.039]; when stratified by histology, the survival advantage was maintained in the SCC group (P=0.007), and lost in the ADC group (P=0.710) (27). These variances in results between these studies may be attributed to factors such as the threshold used to ascertain positivity for PD-L1, application of different antibodies in IHC studies and also possibly, the varying sample sizes. Nonetheless, given the diverse results attained by different studies, it is a possibility that the effect of PD-1/PD-L1 in the IME may be influenced by other factors, including the presence and density of TILs, the predominant PD-L (i.e., PD-L1 *vs.* PD-L2) in the IME, the underlying inducer of PD-L1 expression (e.g., oncogenes), the effects of PD-1 stimulation on other immune cells expressing this receptor in the IME, and other factors, some of which are likely to still be unknown (28). It has been suggested that PD-L1 expression in the IME has a dynamic property (28), which may explain studies that have shown patient response to blockade treatment despite tumors staining negative for PD-L1 (29). Therefore, although PD1/PD-L1 expression in the tumor IME appears to be associated with poorer prognosis in NSCLC, the contradicting results from different studies suggest a more complex interaction in the IME, which warrants further studies to fully characterize the effects of PD-1/PD-L1 in the IME and any other factors that may modify its effects (*Table 1*).

Cytotoxic T-lymphocyte antigen 4 (CTLA-4)

CTLA-4 is a cell-surface molecule found on T-cells, involved in downregulating the activation of T-cells and subsequently is most highly expressed, albeit transiently, in stimulated T-cells, and poorly expressed in resting/non-stimulated T-cells (30). CTLA-4 is an analogue of the co-stimulatory molecule CD28, which is constitutively present on T-cells; CTLA-4 binds to B7 of antigen-presenting cells, causing the activation of T-cells, but has a greater affinity for B7 than does CD28. While CD28 is constitutively active, CTLA-4 only becomes expressed following activation of T-cells (31); however, it is constitutively active only in a subset of T-cells, specifically the T-regs (32). Knowing that CTLA-4 downregulates effector T-cell activation, it follows that a high expression of CTLA-4 on T-cells would lead to a suppressed immune response, useful for peripheral immune tolerance, but disadvantageous for antitumor response (33,34). Increased CTLA-4 in TILs has been associated with mutations of tumor-suppressor genes, specifically the loss of Lkb-1 and kras mutation (35). The notion that CTLA-4 is involved in suppressing antitumor response is supported by several studies that have focused on the resulting antitumor response following CTLA-4 blockade in human melanoma (36), and murine experiments (37). It has been suggested that CTLA-4 is able to promote tumorigenesis, possibly by inhibiting the antitumor cytokine, IL-2. Although IL-2 has T-cell growth-promoting functions, it

Table 1 PD-1/PD-L1 in the immune microenvironment of stage I lung cancer

Author	Sample size	Stages	Conclusion
He *et al.* (10)	139	I	Increased expression in NSCLC is associated with shorter DFS and OS
			PD-L1–/OX40– tumors were significantly associated with longer OS and DFS, compared with PD-L1+/OX40+, or PD-L1/OX40+; P=0.0001
			3 years OS rate in stage IA (P<0.0001):
			❖ PD-L1+/OX40+ =58%
			❖ PD-L1/OX40+ =60%
			❖ PD-L1–/OX40– =72%
			❖ A similar pattern was seen in stage IB tumors and was equally significant (P<0.0001)
			3 years DFS rate in stage IA (P<0.0001):
			❖ PD-L1+/OX40+ =30%
			❖ PD-L1/OX40+ =58%
			❖ PD-L1–/OX40– =62%
			❖ A similar pattern was seen in stage IB tumors and was equally significant (P<0.0001)
Sepesi *et al.* (22)	113	I	Increased expression in NSCLC is associated with shorter OS
			5-year OS rate for:
			❖ High expression of PD-L1 in the TIM and tumor: 20%
			❖ High expression in either TIM or tumor: 76%
			❖ Low expression in the tumor and TIM: 95%
Paulsen *et al.* (27)	536	I–IIIA	Increased stromal PD-L1 expression in NSCLC is associated with improved survival
			5-year DSS rate for:
			❖ High stromal PD-L1 tumors =67% (median DSS: 235 months)
			❖ Low stromal PD-L1 tumors =53% (median DSS: 73 months); P=0.004
			5-year survival rate for:
			❖ High stromal PD-L1 tumors =59% (median: 87 months)
			❖ Low stromal PD-L1 tumors =48% (median 44 months); P=0.039

PD-L1, programmed-death ligand 1; PD-1, program death protein 1; NSCLC, non-small-cell lung cancer; DFS, disease-free survival; OS, overall survival; PD-L1–/OX40–, PD-L1 and OX40 negative; PD-L1+/OX40+, PDL-L1 positive and OX40 positive; PD-L1/OX40+, PD-L1 or OX40 positive; TIM, tumor-infiltrating macrophages; DSS, disease-specific survival.

has also been implicated in promoting T-regs, which have protumor effects and constitutively express CTLA-4. Thus blocking CTLA-4 diminishes tumorigenesis, at least partly by promoting IL-2 while also blocking protumor effects of associated T-regs (*Figure 2*) (38,39). The role of CTLA-4 in LC has been depicted by a number of studies, including those that have shown that increased tumoral CTLA-4 is associated with tumor size, according to the TNM staging (40) and poorer survival (41). Antczak *et al.* showed in their genetic analysis of the expression of CTLA-4 using RNA from resected NSCLC tissues, that CTLA-4 was more greatly expressed in T2 tumors (73%) than T3 and T4 combined (58%), P=0.007; however, interpretation of these results should be done with caution, given the small sample size (n=71), and the fact that the majority of patient samples were classified as T2 (n=33) (40). Deng *et al.* carried out a genetic analysis study on 1,715 patient data extracted from caArray, TCGA and Gene Expression Omnibus (GEO) data repositories. When data from the entire cohort was analyzed, increased CTLA-4 expression trended with worse

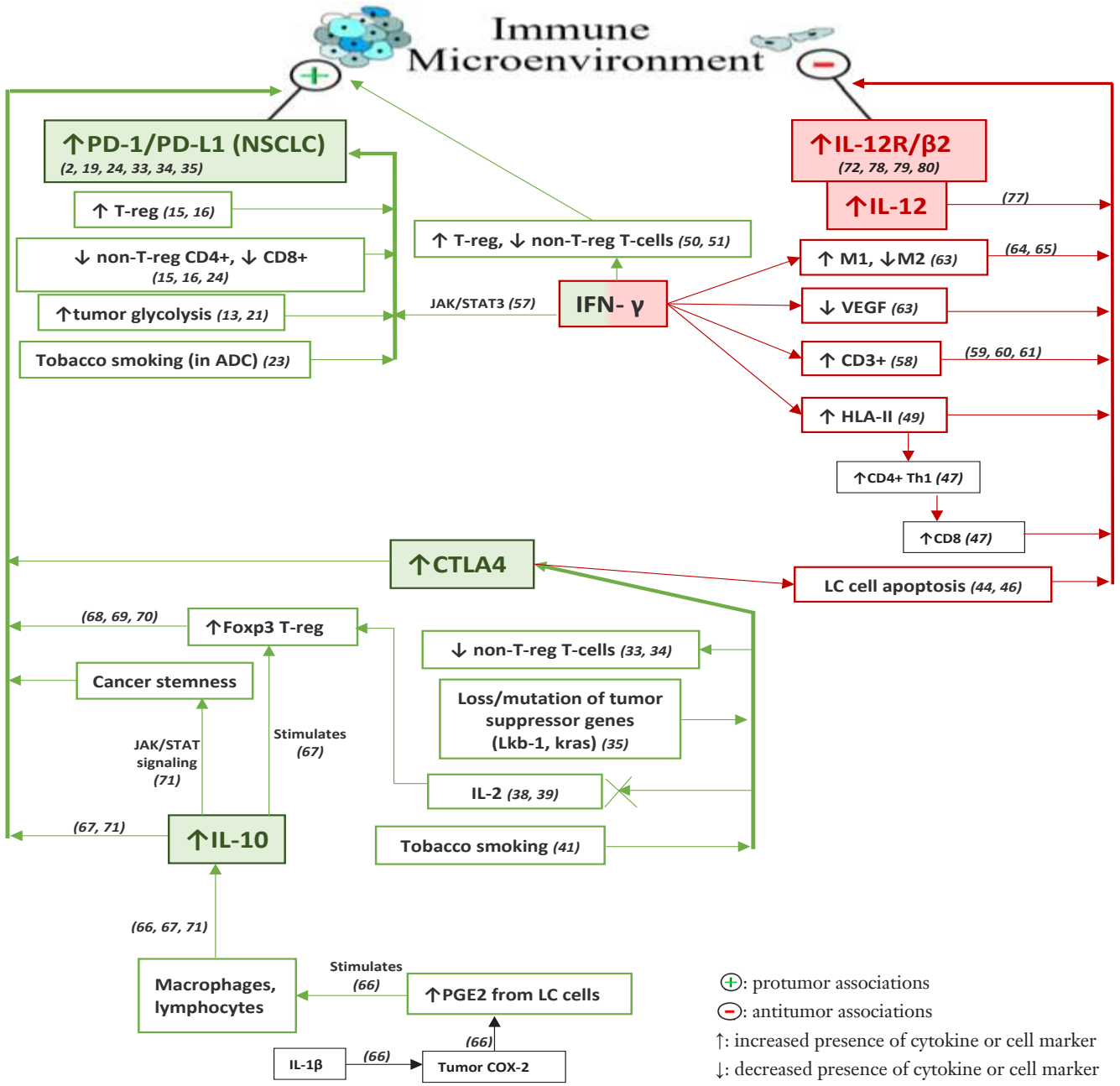

Figure 2 Summary of the prognostic significance of cell markers and cytokines in the immune microenvironment of lung cancer.PD-1, programmed-death protein 1; PD-L1, programmed-death ligand 1; NSCLC, non-small cell lung cancer; T-reg, regulatory T-cell; ADC, adenocarcinoma; SCLC, small cell lung cancer; JAK/STAT, Janus kinase/signal transducer and activator of transcription; M1, macrophage subset M1; M2, macrophage subset M2; IFN-γ, interferon gamma; VEGF, vascular endothelial growth factor; HLA-II, human leukocyte antigen class II; CTLA-4, cytotoxic T-lymphocyte antigen-4; IL-12R/β2, interleukin 12 receptor/beta 2 subunit; COX2, cyclooxygenase 2; PDE2, prostaglandin E-2.

OS; specifically, the high-expression group had a 50-month survival probability of about 58%, compared with the low-expression group with slightly above 60% (P=0.07, 95% CI: 0.99–1.34, HR: 1.15); when only data from patients with sufficient data for multivariate analysis was analyzed (n=412), this trend became significant (95% CI: 2.34–5.11; HR: 3.46). Higher expression of CTLA-4 was also seen in SCC compared with ADC (median expression in SCC and ADC was 111 and 62, respectively; P<0.01). Furthermore, a higher expression of CTLA-4 was shown to significantly correlate with worse OS in ADC but not SCC patients (ADC: 95% CI: 1.16–2.13, HR: 1.57; SCC: 95% CI: 0.7–1.19; HR: 0.91), and when only stage I LC patient data was analyzed, those with a higher expression of CTLA-4 were shown to have worse OS compared with those with lower CTLA-4 expression (95% CI: 1.56–2.90; HR: 2.12). The study further found a significant association between increased CTLA-4 expression and smoking status, in that current/previous smokers had a greater median expression (median expression: 152) than never-smokers (median expression: 59, P<0.01). Increased expression of CTLA-4 also positively correlated with TNM staging, with higher stage being associated with higher CTLA-4 expression; stage I, II, and III patients had median CTLA-4 expression of 80, 107 and 115, respectively; P<0.01 (41). CTLA-4 blockade has been shown to be beneficial in human cancers (42,43). Findings in LC have been contradictory to the premise that CTLA-4 is an immunosuppressive cell marker that diminishes effector T-cell function. A study by Salvi *et al.* on radically resected stage I–III NSCLC using tissue samples from 81 patients and staining by IHC techniques, showed that tumors highly expressing CTLA-4 were associated with a better 5-year survival. Five-year OS rate for high-expression *vs.* low-expression tumors was 64.8% *vs.* 45.9%, respectively; P=0.078 (44). This study is further supported by another study that measured the expression of various immune-related genes in a sample of 178 stage I–IIIA NSCLC patients. The results of this study showed that high expression of CTLA-4 was associated with an improved DFS (81.2 months), compared with low CTLA-4 expression (18.2 months, P=0.012). At 60 months, the OS for the high- and low-expression groups was about 73% and 38%, respectively; P=0.003 (45). Possible explanations that may be given to justify the positive prognosis that appears to be associated with high expression of CTLA-4 in the IME, may include the idea that CTLA-4 may be able to induce apoptosis of LC cells (44,46). However, the results of the aforementioned studies make it apparent that there is no general consensus on the prognostic effects of CTLA-4 in the IME of early-stage LC. Further research may be needed to clarify the prognostic effects of CTLA-4 in LC (*Table 2*).

Human leukocyte antigen (HLA) class II

HLA class II [i.e., major histocompatibility complex (MHC) II], a human antigen found on antigen presenting cells such as B-cells, macrophages, dendritic cells and Langerhans cells, are an essential component of the immune system, involved in presenting exogenous peptide antigens to helper T-cells, initiating an immune response. Several studies have shown an increased expression of HLA-II on TCs and TILs, to be associated with a better prognosis, and a lower disease stage. Zhang *et al.* specifically showed a positive correlation between tumor HLA-DQ expression and DFS in early-stage lung ADC (47). This study carried out 2 separate analyses using data from stage I–III lung ADC patients from their institution (n=165), and similar data extracted from TCGA (n=233). HLA-II was stained for using IHC, and lymphocyte infiltration was determined by genetic analysis. The findings included a statistically significant correlation between high HLA-DQB1 expression and tumor stage, in which the majority of stage I samples had high HLA-DQB1 expression (85.1%), compared with stage II–IIIA where 14.29% expressed high HLA-DQB1; P=0.019; however, this correlation was not seen when the TCGA data was analyzed (P=0.912). Furthermore, HLA-DQB1 was shown to be an independent prognosticator for DFS in stage I lung ADC (HR: 0.686, 95% CI: 0.542–0.868, P=0.002) and non-stage I early-stage lung ADC (HR: 0.875, 95% CI: 0.768–0.996, P=0.044). In the institutional cohort, the recurrence rate in patients expressing high HLA-DQB1 in TCs was 34%, compared with 68% in patients expressing low tumoral HLA-DQB1; P<0.001. Furthermore, high HLA-DQB1 expression was concurrently associated with high lymphocyte (non-specified) infiltration, as well as high CD4+ and CD8+ T-cell expression (P<0.0001). A >10% CD4+ infiltration was seen in 69% of the high HLA-DQB1 group, compared with 43% in the low HLA-DQB1 group; similarly a >10% CD8+ infiltration was seen in 33% of the high HLA-DQB1 group, compared with only 8% in the low HLA-DQB1 group (47). These findings support the notion that increased expression of HLA-II in the tumor IME is associated with a robust antitumoral immune response. The mechanisms underlying such prognostically favorable effects may be associated with the activation of the Th1 subset of helper T-cells, which go on to activate CD8+

Table 2 CTLA-4 in the immune microenvironment of stage I lung cancer

Author	Sample size	Stages	Conclusion
Antczak *et al.* (40)	71	I–IIIB	CTLA-4 expression was greater in T2 tumors (73%), than T3 and T4 combined (58%); P=0.007
Deng *et al.* (41)	1,715	I–IV	Increased CTLA-4 expression trended with decreased OS in the entire cohort (n=1,715) 50-month survival probability in: ❖ High-expression group =58% ❖ Low-expression group =slightly over 60%; (P=0.07, 95% CI: 0.99–1.34, HR: 1.15) Increased CTLA-4 expression significantly correlated with worse OS in the subgroup of patients with sufficient data for multivariate analysis (n=412) ❖ 95% CI: 2.34–5.11; HR: 3.46
Usó *et al.* (45)	178	I–IIIA	High expression of CTLA was associated with improved DFS and OS 60 months DFS rate in: ❖ High-expression group =58% (median DFS: 81.2 months) ❖ Low-expression group =20% (median DFS: 18.2 months); P=0.002 60 months OS rate in: ❖ High-expression group =73% ❖ Low expression group =38%; P=0.003

DFS, disease-free survival; CTLA-4, cytotoxic T-lymphocyte antigen-4; T2, T3, T4, tumor size according to TNM staging; OS, overall survival; CI, confidence interval; HR, hazard ratio.

T-cells to target and destroy TCs (47). He *et al.* generated somewhat corroborating results to that presented by Zhang *et al.* in their retrospective analysis of 139 NSCLC samples, and 139 LC cell lines including NSCLC and small-cell lung cancer (SCLC). HLA-II was more greatly expressed on TCs of stage I and II (35.5% TCs had positive expression), compared with stage III and IV tumors (19.6% TCs had positive expression), albeit insignificantly (P=0.077). There was also a similar, but significant association with TILS; 46.2% stage I–II tumors had positive HLA-II expression (HLA-II+) on TILs, compared with 26.1% in stage III–IV (P=0.023). A positive correlation between HLA-II expression on TCs and TILs (P=0.023) was also shown in this study (48). Furthermore, while there was no correlation with HLA-II expression on TCs and DFS (P=0.642), or OS (P=0.083), a positive correlation was found between DFS and OS, and TILs' expression of HLA-II. DFS in tumors with HLA-II+ TILs was 2.98 years, compared with 1.05 years in tumors with negative HLA-II (HLA-II–) expression on TILs; P=0.028. Similarly, OS was 3.23 years in the HLA-II+ TILs group, *vs.* 1.39 years in the HLA-II– TILs group; P=0.014. A higher HLA-II expression, specifically on TILs, had a greater correlation with NSCLC (54.69%) than SCLC (15.3%); P<0.001 (48). This study might have been limited by its retrospective nature, and the fact that there did not appear to be a standardized method for determining MHC-II positivity. The expression of MHC-II can be induced by interferon gamma (IFN-γ), which concurrently also has immunosuppressive effects that might reverse its antitumoral effects in promoting MHC-II expression (*Figure 2*) (49). For example, IFN-y induces the expression of certain enzymes and molecules that are involved in inhibition of non-T-reg T-cells, while promoting the activity of T-regs (50,51). This mechanism of both inducing antitumoral pathways, while also activating immunosuppressive pathways may be one of the mechanisms underlying why the immune system on its own is unable to eliminate cancers. The lack of expression of MHC-II on SCLC TCs and a lower expression on TILs in SCLC compared with NSCLC may be one of the mechanisms underlying tumorigenesis and poorer outcome in SCLC patients, compared with NSCLC patients. Another study analyzing data extracted from TCGA by Ma *et al.*, showed that increased MHC II gene expression was

Table 3 HLA II in the immune microenvironment of stage I lung cancer

Author	Sample size	Stages	Conclusion
Zhang *et al.* (47)	165	I–III	Increased HLA-DQB1 on TCs was associated with a lower recurrence rate
			Recurrence rate in:
			❖ High-expression group =34%
			❖ Low-expression group =68%; P<0.001
			Increased HLA-DQB1 on TCs was more greatly associated with stage I ADC than stage II–IIIA
			❖ Expression in stage I ADC =85.1%
			❖ Expression in stage II–IIIA ADC =14.29%; P=0.019
He *et al.* (48)	139	I–IV	Positive HLA-II expression in TCs was more greatly associated with stage I and stage II, *vs.* stage III and IV tumors (P=0.077)
			Positive HLA-II expression in TILs was more greatly associated with stage I and II tumors, *vs.* stage III and IV tumors (P=0.023)
			Positive HLA-II expression on TILs was associated with longer DFS and OS
			❖ DFS in HLA-II+ *vs.* HLA-II– TILs was 2.98 years and 1.05 years, respectively; P=0.028
			❖ OS in HLA-II+ *vs.* HLA-II– TILs was 3.23 years, *vs.* 1.39 years, respectively; P=0.014
Ma *et al.* (52)	NA (data retrieved from TCGA)	NA	MHC II gene expression was associated with a greater probability of survival.
			At 125 months, approximate survival probabilities for:
			❖ Group with upregulated MHC II gene expression =100%
			❖ Group with downregulated MHC II gene expression =75%; P<0.0001

ADC, adenocarcinoma; HLA, human leukocyte antigen; TC; tumor cell; TIL, tumor-infiltrating lymphocyte; DFS, disease-free survival; OS, overall survival; NA, not applicable or data not available.

associated with a more favorable prognosis, specifically, a greater probability of survival (P<0.0001). At 125 months, survival probability was approximately 75% in the group in which MHC-II genes were downregulated, compared with 100% in the group in which MHC-II was upregulated (52). In summary, it appears that increased MHC-II expression in the IME may be a positive prognosticator for recurrence and survival. Therefore, MHC II may not only serve as a prognostic marker, but also a potential point for therapeutic exploration. Further research into pure inducers of MHC-II in the tumor microenvironment might be one such therapeutic point for exploration (*Table 3*).

Cytokines

This section will focus on the findings relating to the functions and prognostic value of various cytokines in the IME of stage I and early-stage LC. Cytokines that will be discussed include: IFN-γ, interleukin 10 (IL-10), interleukin 12 (IL-12), interleukin 37 (IL-37) and CCL20.

IFN-γ

IFN-γ is a cytokine that promotes inflammation and is elaborated by immune cells including macrophages, T-cells and NK cells. IFN-γ signaling pathway is involved in modulating the responses of the innate and adaptive immune systems to infections (viral and bacterial), inflammation and apoptosis (53). It has been established that IFN-γ is essential not only for response to infections, but also for eliminating cancer. Murine studies by Ligocki *et al.* have shown that IFN-γ knockout (KO) mice experienced progressive growth of introduced intraocular tumors (54). Due to the proinflammatory nature of IFN-γ, it can reasonably be assumed that a high expression of IFN-γ in the tumor environment may be associated with a robust antitumoral inflammation and death, with resultant positive prognosis; this assumption is supported by studies that depict the importance of IFN-γ in tumor rejection

(55,56). However, other studies have resulted in results contradicting this basic understanding of the functions of IFN-γ. A study performed by Zhang *et al.* on LC cell lines in which cytokine expression was analyzed by flow cytometry, showed that IFN-γ elaborated by tumor-associated macrophages (TAMs) appears to play a protumor role in the IME of LC. This study specifically showed that IFN-γ acts via the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling pathway to induce the expression of PD-L1, whose protumor roles in NSCLC have been previously discussed in this article. TAMs (at least partially through IFN-γ) were shown to increase the PD-L1 expression in LC cells from a baseline expression of 7.8% to 37.3% (P<0.0001), as well as promote invasiveness as ascertained by an invasion index (P<0.001) (57). A different study by Gao *et al.*, who used 50 resected treatment-naïve locally advanced (IIIA) lung ADC tissues showed contradicting results. This study showed that increased IFN-γ expression is associated with increased CD3+ T-cells within the tumor bed (n=26, P=0.039); however, the specific subset of T-cells elaborated was not specified (58). Nonetheless, this finding supports the notion that IFN-γ plays an antitumoral role in the IME, given that various other studies have shown that increased total T-cell infiltration in the IME is associated with favorable prognosis including reduced recurrence (59-61) and improved DFS (60). Gao *et al.* also showed a positive correlation between IFN-γ and PD-L1 expression (n=17, P=0.005), which corroborates the aforementioned study by Zhang *et al.*, in which IFN-γ was shown to increase PD-L1 in LC cells. However, this study further showed that IFN-γ expression was associated with an increased DFS, albeit insignificantly, which contradicts the study by Zhang *et al.*, in which IFN-γ was shown to promote tumor invasiveness (57). Specifically, the study by Gao *et al.* showed the median DFS is IFN-γ positive patients to be 22.28 months (95% CI: 12.42–32.13 months), compared with the IFN-γ negative group with a median DFS of 12.78 months (95% CI: 10.37–15.19; P=0.125) (58). Although the proinflammatory functions of IFN- γ are well established, these contradictory results put into question whether it truly is antitumoral. Studies have shown that IFN-γ pathways are inactivated in cancers, including lung ADC cell lines (62), which supports the notion that the presence of active IFN-γ in the IME may play an antitumoral role. A murine study by Ren *et al.* showed that IFN-γ administration to LC murine models significantly reduced tumor weight by 26% compared to the control group, which was administered saline (P<0.01). Tumor weight was also decreased by the cycloxygenase-2-specific non-steroidal anti-inflammatory drug, celecoxib (31% decrease, P<0.01), and even more so when both IFN-γ and celecoxib were combined (34% decrease in tumor weight, P<0.01) (63). Further findings of this study support that IFN-γ likely plays an antitumoral role in the IME. Specifically, IFN-γ alone, and also in combination with celecoxib, increased M1 macrophage population by 116% (P<0.01), and also decreased M2 macrophage population by 48% (IFN-γ alone) and 52% (IFN-γ in combination with celecoxib), compared with the control group (P<0.01) (63). These findings support an antitumoral response, since M1 macrophages have been suggested to be associated with antitumoral response (64) while M2 macrophages have been shown to be protumor (64), and also to be involved in the promotion of metastasis in early-stage (I–IIIA) LC (65). Not only does this macrophage association with IFN-γ possibly explain an antitumor response, the finding that IFN-γ decreases VEGF and microvessel proliferation also supports this notion, as this suggests that IFN-γ may be associated with starving TCs of nutrients, promoting TC death (63). Given the contradictory results from various studies, the prognostic value of IFN-γ in the IME of early-stage LC remains uncertain, and further prospective studies using human LC tissues are still required (*Table 4*).

IL-10

Huang *et al.* performed a study on human lung ADC cell lines, which attempted to expound on the elaboration of various cytokines by LC cells. This study showed that IL-1β increased prostaglandin E2 (PGE2) by TCs in a dose-dependent manner via a cycloxygenase-2 (COX-2)-based mechanism, which then stimulates IL-10 expression by macrophages and lymphocytes (66). A study by Vahl *et al.* performed using IHC on formalin fixed, paraffin embedded tissue samples, polymerase chain reaction (PCR) and flow cytometry, showed that the presence of IL-10 in the region around the tumor (as opposed to within the tumor itself), positively correlated with increased tumor size in stage I–IIIA NSCLC samples; correlation coefficient (r)=0.6, P<0.05. The study further concluded that this IL-10 was elaborated by non-T-lymphocyte cells, including macrophages and other leukocytes. Furthermore, IL-10-receptor (IL-10R) within the tumor positively correlated with tumor size in ADC (r=0.71, P<0.01), and there was similarly an increased number of Fox-p3+ T-regs expressing IL-10R in the tumor, compared with the surrounding

Table 4 IFN-γ in the immune microenvironment of stage I lung cancer

Author	Sample size	Stages	Conclusion
Zhang *et al.* (57)	NA (LC cell lines)	NA	❖ TAMs (at least partially by elaborating IFN-γ) were shown to increase the PD-L1 expression in LC cells from a baseline expression of 7.8% to 37.3% (P<0.0001)
			❖ TAMs (at least partially by elaborating IFN-γ) promote invasiveness as ascertained by an invasive index (P<0.001)
Gao *et al.* (58)	50	Locally advanced (IIIA)	❖ Increased IFN-γ is associated with increased CD3+ T-cell (n=26, P=0.039)
			❖ Increased IFN-γ is associated with increased PD-L1 (n=17, P=0.005)
Ren *et al.* (63)	NA (murine study)	NA	❖ IFN-γ administration to murine LC models led to a decrease in tumor weight by 26% when administered alone, compared with control group (saline administered) (P<0.01)
			❖ IFN-γ administration increased M1 macrophages by 116% compared with control (P<0.01)
			❖ IFN-γ administration decreased M2 macrophages by 48% compared with control (P<0.01)

IFN-γ, interferon gamma; TAM, tumor-associated macrophages; PD-L1, programmed-death ligand 1; LC, lung cancer; NA, not applicable or data not available.

non-tumoral regions (67). These findings support the notion that IL-10 promotes tumorigenesis, and one of the mechanisms by which this may be accomplished is by stimulating T-regs, which have been shown to promote tumor growth and survival, with subsequent negative prognosis in NSCLC (68-70). Another study applying PCR in 102 NSCLC samples corroborated the findings of Vahl *et al.*, in that this study showed that IL-10 was elaborated by TAMs. This study further showed a positive correlation between IL-10R and JAK1 signaling, indicating that IL-10 maybe be associated with growth promotion in NSCLC. Specifically, the findings of the study provided a conclusion that IL-10 mediates stem-cell-like properties in cancer (i.e., cancer stemness) via the JAK/STAT signaling pathway (71). These findings were further supported by the same study which showed that both IL-10 and IL-10R mRNA were more significantly expressed in stage III tumors than in stage I and II tumors (P<0.05). The expression values of IL-10 in stage I, II and III were approximately 5, 10 and 15, respectively; similarly, values for relative expression of IL-10R were approximately 2, 4 and 6, respectively. Furthermore, high expression of both IL-10 and IL-10R was associated with worse prognosis in patients, specifically a worse DFS. Survival curves showed a distinctly greater DFS in the group with low IL-10 expression compared with the high-expression groups. At 15 months, DFS for the low-and high-expression groups was approximately 80% and 40%, respectively (P<0.001) (71). In contrast to these aforementioned studies, Usó *et al.* showed in their study of gene expression of immune-related genes in 178 stage I–IIIA resected NSCLC samples that increased levels of IL-10 was associated with improved DFS and improved OS. DFS was 49.3 months in high-expression group, versus 18.8 months in the low-expression group; P=0.029; similarly, OS was 81.2 months in the high-expression group, compared with 37 months in the low-expression group; P=0.03 (45). These results differ from the finding by Yan *et al.*, in which there was no correlation between IL-10 expression and either DFS (P=0.575), or OS (P=0.644) (72). Another study by Wang *et al.* corroborates the earlier mentioned studies, in that the findings of this study support the notion that IL-10 is a protumor cytokine that promotes tumorigenesis and invasiveness. In this study, TAMs isolated from 63 NSCLC samples were analyzed for expression of IL-10 by IHC and real-time PCR (RT-PCR). Increased IL-10 expression by TAMs was shown to be associated with late-stage disease (II–IV), compared with early-stage disease (stage I); P=0.016. Furthermore, increased IL-10 was also associated with invasive parameters including lymph node metastasis (P<0.001), pleural invasion (P=0.002), and poorly differentiated (compared with well- or moderately differentiated) tumors (P=0.001). Based on the findings of these studies, it appears that IL-10 via

Table 5 IL-10 in the immune microenvironment of stage I lung cancer

Author	Sample size	Stages	Conclusion
Vahl *et al.* (67)	48	I–IIIA	❖ IL-10 expression in the peritumoral region correlated positively with increased tumor size (r=0.6, P<0.05) ❖ IL-10R within ADC tumors correlated positively with tumor size (r=0.71, P<0.01)
Yang *et al.* (71)	102	I–IV	❖ IL-10 promotes JAK/STAT signaling ❖ IL-10 and IL-10R correlated with tumor stage—they were more greatly expressed in stage III than stage I and II tumors (P<0.05) ❖ The expression values of IL-10 in stage I, II and III tumors were approximately 5, 10 and 15, respectively. Values for relative expression of IL-10R in stages I, II and III were approximately, 2, 4 and 6, respectively ❖ IL-10 and IL-10R were associated with worse DFS: 15 months DFS was 80% and 40% in the low- and high-expression groups, respectively (P<0.001)
Usó *et al.* (45)	178	I–IIIA	Increased levels of IL-10 were shown to be associated with improved DFS and OS DFS in: ❖ High-expression group =49.3 months ❖ Low-expression group =18.8 months; P=0.029 OS in: ❖ High-expression group =81.2 months ❖ Low-expression group =37 months; P=0.03
Yan *et al.* (72)	107	I–IV	No correlation between IL-10 expression and either DFS (P=0.575), or OS (P=0.644)
Wang *et al.* (73)	63	I–IV	Increased expression of IL-10 by TAMs was associated with: ❖ Late-stage disease (II–IV), compared with early stage disease (stage I) (P=0.016) ❖ Lymph node metastasis (P<0.001) ❖ Pleural invasion (P=0.002) ❖ Poorly differentiated (compared with well- or moderately differentiated) tumors (P=0.001)

IL-10, interleukin-10; IL-10R, interleukin-10 receptor; r, correlation coefficient; ADC, adenocarcinoma; JAK/STAT, Janus kinase/signal transducer and activator of transcription; DFS, disease-free survival; OS, overall survival; TAM; tumor-associated macrophages.

various mechanisms probably supports tumor growth and invasion in NSCLC (73) (*Table 5*).

IL-12

IL-12 is a proinflammatory cytokine released by antigen presenting cells such as dendritic cells and phagocytes, and it causes the differentiation of T-helper cells into the Th1 subset, with subsequent release of IFN-γ that results in a cell-mediated immune response (74) The IL-12 receptor is composed of two beta subunits: β1 and β2; both of these subunits are needed for high-affinity binding of the cytokine (75). Murine studies have shown that IL-12-receptor β2 subunit (IL12R/β2) KO mice were more susceptible to developing tumors (76), and administration of IL-12 reduced recurrence of melanoma tumors in murine models (77). These findings suggest that the loss of IL-12R/β2 may be a factor associated with tumorigenesis. This notion is supported by another study that showed that while normal peritumoral lung tissue expressed IL-12R/β2, only 41% of lung ADCs expressed the receptor subunit (78). This study analyzed tissue samples from 70 resected lung ADCs using IHC. Another finding of the same study was that IL-12R/β2 expression was greater in stage I lung ADC than in stage II–III combined (P=0.012), further supporting the notion that abrogation of IL-12R/β2 plays an important role in tumorigenesis and possibly even invasiveness. Other

Table 6 IL-12 in the immune microenvironment of stage I lung cancer

Author	Sample size	Stages	Conclusion
Airoldi *et al.* (79)	70	I–IIIA	IL-12R/β2 is less expressed in lung ADCs (41%), compared with normal peritumoral lung tissue, in which it is always expressed
			IL-12R/β2 was more greatly expressed in stage I ADC than in stage II and III combined (P=0.012)
Suzuki *et al.* (80)	956	I	High expression of IL-12R/β2 is associated with a greater recurrence-free probability
			Recurrence-free probability in:
			❖ High-expression group =90%
			❖ Low-expression group =80%; P=0.026
Yan *et al.* (72)	107	I–IV	❖ An insignificant positive correlation between high expression of IL-12R and DFS was found
			❖ HR: 1.285; 95% CI: 1.160–1.966; P=0.206

IL-12, interleukin-12; IL-12R/β2, interleukin-12 receptor/beta-2 subunit; ADC, adenocarcinoma; DFS, disease-free survive; HR, hazard ratio; CI, confidence interval.

laboratory studies have depicted the antitumorigenic effects of IL-12, such as that by Airoldi *et al.*, in which KO mice were inoculated with IL-12R/β2-positive (IL-12R/β2+) melanoma cells, and then subsequently administered IL-12, or a placebo. The IL-12-administered group showed a significant reduction in tumor size, compared with the group administered the placebo (P=0.0286) (79). A retrospective analysis using tissue microarray and IHC on 956 stage I lung ADCs, showed that a high expression of IL-12R/β2 was associated with better prognosis; this was noted as a reduced risk of recurrence (5-year recurrence-free probability was 90% in the high-expression group, compared with 80% in the low-expression group; P=0.026) (80). Improved DFS was depicted by Yan *et al.* in their IHC study of 107 resected stage I–IV NSCLC tissues, although this was an insignificant finding. High expression of IL-12R was associated with a longer DFS (HR: 1.285; 95% CI: 1.160–1.966; P=0.206) (72). These results suggest that IL-12R/β2 could possibly be exploited as a therapeutic target in LC (*Table 6*).

Interleukin-37 (IL-37)

IL-37 is a cytokine that belongs to the interleukin-1 (IL-1) family, which has been shown to have immunosuppressive and anti-inflammatory functions (81-83). One study on 182 stage I–III NSCLC patients, performed by IHC, RT-PCR and western blotting, to detect IL-37 protein and mRNA in tissues samples, showed that IL-37 protein and also mRNA were more significantly expressed in normal lung tissue (58.2%) than in NSCLC (41.8%) tissues; P<0.01. This lower intratumoral expression was also associated with shorter OS in tumors with low IL-37 expression, compared with NSCLC tissues with higher expression of IL-37 protein and mRNA (P<0.001). Furthermore, IL-37 was more highly expressed in early-stage (I–II) tumors than stage III tumors (P=0.000) (84). Based on these results, it is reasonable to assume that IL-37 expression appears to be protective against carcinogenesis and tumor progression in LC. This is further corroborated by experimental data showing that IL-37 suppresses tumor growth in mice models (85). There have been a number of postulated mechanisms by which IL-37 is able to potentially inhibit tumorigenesis, including recruitment of NK cells (86), inhibition of growth-promoting pathway (87), and also possibly downregulating VEGF to diminish tumor vascularization (84).

CCL20

CCL20 is a chemokine that is chemoattractive for lymphocytes, and acts on its receptor, CCR6 (88). LC appears to stimulate increased expression of CCL20 (89). Mony *et al.* performed a study using microarray datasets extracted from GEO (n=239), which showed that increased expression of CCL20 was associated with a lower recurrence risk in early-stage LC (P=0.0012) (68). A relatively small study by Kirshberg *et al.* (90) on the expression of CCR6/CCL20 in NSCLC and the possible relationship to prognosis showed that NSCLC samples highly expressed CCL20, compared with adjacent normal lung tissue, similar to the findings by Zhang *et al.* (89). Zhang *et al.* performed

a study using samples from 162 patients with stage I and II lung ADC; CCL20 was stained for by IHC, and RNA was also extracted from tissue samples to assess CCL20 expression. As previously mentioned, the study found little-to-no CCL20 expression in lung tissue adjacent to the cancer, while cancer tissues appeared to express CCL20. Furthermore, the findings in terms of the relationship between CCL20 expression and recurrence contradicts the findings by Mony *et al*, in that samples taken from patients who experienced recurrence expressed higher levels of CCL20 (>50% of cells stained for CCL20 in 76% of patient samples), compared with the non-recurrence group, in which high-expression was only 8%; P<0.001. Similarly, mRNA levels for CCL20 were also more greatly expressed in the recurrence, *vs.* the non-recurrence group (P<0.001) (89). In the study by Kirshberg *et al.*, the majority of tumor samples highly expressed (>50%) CCL20 (78%), while only a minority of tumor samples highly expressed (>50%) CCR6 (16.5%). Normal lung tissue stained negatively for CCL20 compared with lung ADC tissues which stained positively. This study showed a significant negative correlation between high CCR6 expression and shorter DFS (P=0.0076; 95% CI: 1.52–15.563); they concluded that high expression of CCR6 was associated with a 4.87-fold increased risk of recurrent disease and was independently associated with a shorter DFS. The study was performed on 49 stage I–III lung ADC tissue samples; CCL20/CCR6 was stained for by IHC. Further analysis using NSCLC cell lines showed that stimulation of the CCL20/CCR6 pathway leads to NSCLC proliferation (90). Although the sample size in this study was small, further studies have supported their findings that CCR6/CCL20 expression is associated with carcinogenesis and proliferation in LC (89,91). These findings suggest a possible therapeutic target for LC, and there are a number of studies based on this concept (92,93).

Conclusions

In summary, OX40 expression appears to be a poor prognosticator for OS and DFS, despite other studies on other human cancers finding this cell marker to be associated with improved survival and non-recurrence advantages. More studies may be warranted to assess the relevance of OX40 expression in the IME of NSCLC. The studies reviewed with regard to the relevance of PD-L1 in the IME of early-stage LC contradicted one another in their findings. While most studies on NSCLC seemed to indicate that PD-L1 expression is associated with worse DFS, OS and disease stage, the largest study with over 500 samples analyzed, indicated that PD-L1 expression in NSCLC was associated with improved survival, albeit, this finding appeared to only be statistically significant for SCC and not ADC. The studies on CTLA-4 reviewed in this paper similarly gave contradictory results, in which CTLA-4 was associated with increased survival in one study and decreased survival in another. Based on the function of CTLA-4 as a down-regulator of T-cell activation, it may be logical to conclude that high expression would be a negative prognostic factor; however, further research is still required to reach a conclusion on the effects of CTLA-4 in LC. HLA-II expression in TCs, and more so in TILs, has been shown to be associated with prolonged DFS and OS by a number of studies, which is in line with the known functions of HLA-II on APCs, in presenting antigens to helper T-cells. It appears that high HLA-II expression helps to mediate an antitumor response that is prognostically favorable in LC. Although IFN-γ has been shown to be a pro-inflammatory cytokine involved in tumor rejection, findings relating to LC are inconsistent. While one study showed that IFN-γ had a protumor role in the IME of LC via stimulating growth pathways such as the JAK/STAT pathway, another study showed that IFN-γ promoted an increase in total T-cell (CD3+), supporting the notion that increased T-cell infiltration of tumors is associated with an antitumoral response. Another LC study using murine models showed that IFN-γ decreased tumor mass, possibly by elaborating M1 macrophages and decreasing M2 macrophages and VEGF. Further studies focused on human LC tissue samples may be warranted to determine with certainty, the prognostic significance of IFN-γ in the IME of LC. Findings of various studies have indicated that the loss of function of IL-12 in the tumor IME may be associated with tumorigenesis and progression of tumors, while the presence of IL-12 is associated with the opposite, specifically favorable non-recurrence. IL-37 expression has been shown to be associated with improved OS and early-stage disease, as opposed to late-stage disease, which may be mediated by mechanisms such as inhibiting growth pathways, downregulation of VEGF leading to negative regulation of angiogenesis, and also by NK cell recruitment. Finally, CCL20 stimulation in the tumor IME appears to be prognostically unfavorable in LC. While one study showed increased expression to be associated with lower recurrence, another study supported by other studies showed consistently that increased CCL20 expression was

associated with higher recurrence. The knowledge of the roles of cytokines and cell surface markers in the IME of LC, provides opportunities for the conception of prognostic scores that could potentially be used in the future to stratify patients based on risk and into groups that may benefit most from neoadjuvant and/or adjuvant therapy. Better characterization of the true IME including the tumor, and the tumoral and peritumoral stroma, is currently most suitably achieved through analysis of resected specimen. Further research is still needed to clarify the roles of some cytokines and cell markers in the IME and their clinical usefulness as prognosticators.

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