MINI REVIEW

Tumor-infiltrating B cells: their role and application in anti-tumor immunity in lung cancer

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Evidence indicates that lung cancer development is a complex process that involves interactions between tumor cells, stromal fibroblasts, and immune cells. Tumor-infiltrating immune cells play a significant role in the promotion or inhibition of tumor growth. As an integral component of the tumor microenvironment, tumor-infiltrating B lymphocytes (TIBs) exist in all stages of cancer and play important roles in shaping tumor development. Here, we review recent clinical and preclinical studies that outline the role of TIBs in lung cancer development, assess their prognostic significance, and explore the potential benefit of B cell-based immunotherapy for lung cancer treatment.

Keywords: lung cancer; B cells; tumor-infiltrating B cells; Bregs; immunotherapy

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INTRODUCTION

Lung cancer is a heterogeneous malignant disease that is the leading cause of cancer-related deaths worldwide.[1] The growth, invasion and metastasis of lung cancer is a complex and dynamic process that involves both intrinsic genetic abnormalities of the tumor tissue and its interactions with immune cells within the local microenvironment.[2] Approximately two-thirds of lung tumor-infiltrating immune cells are composed of T and B cells, with the remaining cells being composed of tumor-associated macrophages (TAMs) and a small number of infiltrating dendritic cells and natural killer (NK) cells.[3] These tumor-infiltrating lymphocytes (TILs) are involved in the anti-tumor response within the lung tumor niche.[4]

The multifaceted effects of lung cancer-associated T cells have been intensely studied. It has been noted that $CD4^+$ Th1 cells, activated $CD8^+$ T cells, and γδ-T cells are often involved in type I immune responses and associated with favorable prognosis in lung cancer patients,[5,6] whereas Th2, Th17, and $Foxp3^+$ regulatory T (Treg) cells are often associated with tumor progression and unfavorable prognosis.[7] Further, clinical responses observed from immune checkpoint blockade therapies (inhibiting the immunological targets PD1/PD-L1 or CTLA4)[8] and CAR-T cell therapies[9] have spurred great interest in the field of anti-tumor immunotherapy. These T cell-targeted therapies have yielded promising clinical results; however, not all patients respond to these therapies, indicating the need for other therapeutic approaches.

B cells infiltrating lung cancer have their own unique roles in anti-tumor immunity. Recent studies have demonstrated that proliferating B cells can be observed in ~35% of lung cancers.[10] Furthermore, tumor-infiltrating B lymphocytes (TIBs) can be observed in all stages of human lung cancer development,[11] and their presence differs between stage and histological subtypes,[12,13] suggesting a critical role for B cells during lung tumor progression. TIBs participate in both humoral and cellular immunity, but their roles in antitumor immunity remain controversial.[14] Some studies show the capacity of B cells to induce and maintain beneficial antitumor activity, while others have found that B cells may exert protumor functions due to their various immunosuppressive subtypes. Here, we will review the role of TIBs in lung cancers and discuss their potential clinical applications.

THE INFILTRATION, LOCATION, AND MAINTENANCE OF TIBS IN HUMAN LUNG CANCER

The localization of TIBs is highly controlled by the signals or stimuli within the tumor microenvironment. Evidence has shown that B cell infiltration in human lung cancer is significantly higher than that in surrounding tissue or in distant non-tumoral tissue.[15] The presence of high endothelial venule (HEV) may be critical for driving B cell homing and infiltration into the tumor niche via ligand/receptor interactions of PNAd/CD62L, adhesion molecules, and integrins.[16,17] Importantly, the B cell chemoattractant CXCL13, secreted by tumor cells, follicular dendritic cells, and T follicular helper cells in human lung tumor lesions, was shown to be responsible for the influx of B cells into the tumor.[16,18,19] Upon entering the local microenvironment, tumor antigens released from lung cancer cells aid in B cell aggregation and trigger B cell-mediated antigen presentation, which facilitate the maintenance of B cell activation and proliferation.[19,20]

Immunohistochemistry (IHC) and flow cytometric analysis show that all major subsets of B cells can be found within TIB populations (Table 1).[21] TIBs, including $CD20^+$ B cells and $CD138^+$ plasma cells, primarily localize to lymphoid aggregates

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Table 1. Characterization of TIB subsets in human lung cancer

TIB subset	Marker ($CD45^+CD19^+$)	Ref
Germinal center B	$Bcl6^+CD20^+/Ki67^+CD20^+$	21, 22
Naive B	$CD20^+CD38^-CD27^-IgD^+$	21
Switched-memory B	$CD20^+CD38^{+/-}CD27^+IgD^-$	21
Non-switched-memory B	$CD20^+CD38^{+/-}CD27^+IgD^+$	21
Plasma cell	$CD20^-CD38^{++}/CD138^+$	21, 82

within the lung tumor stroma, the typical model being termed a tertiary lymphoid structure (TLS).[10,22] TLSs exhibit features of secondary lymphoid organs with an ongoing immune reaction and exacerbate the local immune responses in chronic inflammatory settings, including tumors.[23] It has been verified that TLSs form an efficient and beneficial immune response in most solid tumors.[24] Germain et al. described this structure in detail within lung cancer and showed compartmentalized B cell-rich (or follicular area) and T cell-rich zones. B cell-rich areas can be further divided into germinal center (GC) cores and mantle regions.[21] Within the GC, TIBs have an increased GC ($Bcl6^+CD20^+$/$Ki67^+CD20^+$) phenotype,[21,22] and B cells proliferate and differentiate into plasma cells. TLS-derived GC-B cells of non-small cell lung cancer (NSCLC) patients expressed activation-induced cytidine deaminase (AID), the enzyme critical for immunoglobulin somatic hypermutation (SHM) and class-switch recombination (CSR).[21,25] At the mantle, naive B cells ($CD20^+CD38^-CD27^-IgD^+$) can be observed surrounding GCs, with switched-memory B cells ($CD20^+CD38^{+/-}CD27^+IgD^-$) and non-switched-memory B cells ($CD20^+CD38^{+/-}CD27^+IgD^+$) found at the interface between the two zones. Plasma cells ($CD20^-CD38^{++}/CD138^+$), primarily long-lived plasma cells, accumulate in the follicular periphery, tumor stroma and fibrotic areas, which is sustained by proliferation-inducing ligand$^+$ ($PRIL^+$) myeloid cells in the lung cancer stroma.[13,26] These observations suggest that B cells can exist in a continuum of naive cells to differentiated plasma cells within the tumor microenvironment.

Importantly, TIBs maintain the structure and function of TLS within the lung tumor microenvironment by secreting cytokines and chemokines. Early studies using murine models have identified CXCL13 and lymphotoxin (LT)[27,28] as two critical factors in the formation, development, and maturation of isolated lymphoid follicles of the gut.[23] Similarly, in human inflamed lungs, such as in chronic obstructive pulmonary disease and lung cancer, B cells produce CXCL13 and LT via toll-like receptor 4 signaling, which positively correlates with the formation and high density of TLS.[29,30] Analysis of the factors secreted by TIB, as shown in Fig. 1, provides evidence of ongoing B cell proliferation and induction of antitumor immunity of TLS. Of note, murine models have shown the presence of TLS and follicular B cells in melanoma and/or pulmonary metastasis via LT.[31,32] However, some premalignant and malignant tissues in mice are poorly infiltrated by B cells.[33,34] The difference in status of TLS-containing follicular B cells in particular experimental settings to some extent may explain why murine models do not recapitulate all clinical-pathological features of human disease.

PROTECTIVE EFFECT OF TIBS FOR ANTI-TUMOR IMMUNITY IN LUNG CANCER

The development of humoral immunity is the primary function of B cells. Within the lung, tumor-associated B cells can differentiate into plasma cells and produce tumor-specific antibodies that recognize and react against tumor-associated antigens, such as LAGE-1, TP53, and NY-ESO-1.[21] Lung cancer patients with a higher proportion of tumor-associated antigen reactive immunoglobulin tend to have a higher density of follicular B cells.[21] Moreover, both follicular B cells and tumor-infiltrating plasma cells are correlated with a better long-term survival of lung cancer patients, indicating the protective role of plasma cells and antibodies in antitumor immunity (see section below for detail).[21,35] In accordance with this evidence, increased tumor B cell-derived IgG in the sera is associated with a significantly higher number of tumor regressors in murine xenograft models of human lung squamous cell carcinoma (SCC) tissue.[36] Furthermore, immunoglobulins secreted by lung tumor-stimulated B cells can mediate tumor lysis by antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).[37,38]

In addition to the production of antibodies, B cells also promote tumoral T cell responses, such as regulation of T cell activation, expansion, and memory formation. Studies have demonstrated that increased levels of $CD8^+$ and $CD4^+$ TILs co-localizing with high $CD20^+$ B cell infiltration are related to long-term survival in NSCLC.[6,39] Further, studies by Eerola et al. showed a significant association between B cells and $CD8^+$ TILs in large cell lung carcinoma (LCC).[40] These data suggest the beneficial effect of TIBs on T cell-mediated antitumor immunity, possibly through antigen presentation by TIBs. Experimental data from studies in human/murine lung tumor models provide support for B cell interaction with T cells. Tumor-stimulated B cells can rapidly increase the expression of HLA class II and the costimulatory molecules CD40, CD80, and CD86.[41–43] Tumor antigen can be presented by TIBs to tumor-infiltrating $CD4^+$ T cells, which leads to $CD4^+$ T cell differentiation and clonal expansion.[41] Specifically, B cells with the $CD69^+$HLA-$DR^+CD27^+CD21^+$ phenotype in NSCLC patients correlated with an effector T cell response (IFN-γ^+ $CD4^+$ TILs).[41] A similar ability of lung TIB cells to induce T cell secretion of IFN-γ has been reported in murine models of lung metastatic tumors.[42] Consistently, evidence suggests that an increase in $CD4^+$ and $CD8^+$ TCR repertoire clonality is closely associated with a high density of follicular B cells within the lung tumor niche.[44]

Although likely not a major mechanism, evidence exists that the activated B cells can directly lyse tumor cells. TIBs have been shown to have cytotoxicity towards hepatoma cells via secretion of granzyme B and TRAIL,[45] and human B cells stimulated with IFN-α or a toll-like receptor 9 agonist produce functional TRAIL that is cytotoxic towards melanoma cell lines.[46] However, IL-21-induced granzyme B-expressing B cells are detrimental, as they can suppress antitumor T cell responses.[47] Figure 1 summarizes the current understanding of the dynamic conversion processes and immunomodulatory effects of B cells in lung cancer. A better understanding of the functional properties of TIBs in lung cancer will help develop improved immunotherapies against lung cancer.

INHIBITORY EFFECT OF BREGS ON ANTITUMOR IMMUNITY IN LUNG CANCER

B cells with tumor-promoting effects have been defined as regulatory B cells (Bregs). Multiple subtypes of Bregs have been identified based on the expression of surface markers, production of soluble factors, and properties of promoting tumor growth in both human and animal tumors. Although diversity of phenotypic markers across different tumors has been reported,[48] the canonical phenotypes in both humans and mice are concentrated in memory $CD27^+$ and transitional $CD38^+$ B cells and share phenotypes with plasma cells, such as IgA^+CD138^+ and IgM^+CD147^+.[49,50] Similarly, in both human and mouse studies, Bregs display their characteristic immunosuppression by secreting cytokines such as IL-10 and TGF-β and/or upregulating immune regulatory ligands such as PD-L1 and CTLA-4, which attenuate T and NK cell responses and/or facilitate the protumor effects of Tregs, MDSCs, and TAMs.[51]

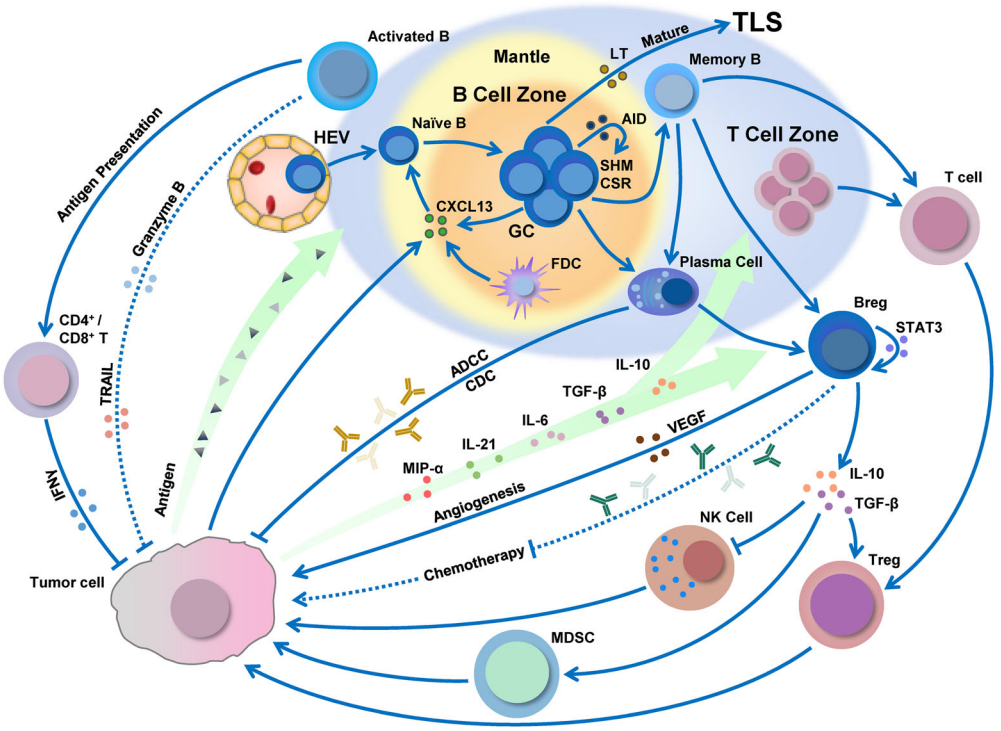

Fig. 1 The dynamic conversion processes and immunomodulatory effects of B cells in lung cancer. B cell infiltration, development, and polarization can be regulated by the tumor microenvironment. B cells not only inhibit tumor growth by secreting immunoglobulins, promoting T cell response, and potentially direct killing tumor cells but can also suppress antitumor immune response by Bregs, which produce immunosuppressive cytokines to regulate T cells, NK cells, and myeloid-derived suppressor cells (MDSC); secrete pathological antibodies; or promote angiogenesis. The large blue area represents the TLS within the lung tumor. Solid lines and dashed lines depict the proved and potential effects of B cells, respectively. Arrows represent promotion effects, and the blunt ends are suppression effects

As part of a continuum of B cell phenotypes, Bregs likely acquire their regulatory phenotype within the lung tumor microenvironment.[52] The polarization of B cells to high IL-10-secreting cells is associated with high expression of inflammatory signals either derived from the tumor directly or indirectly by other microenvironment constituents.[53–56] In one model, LPS-stimulated lung cancer cells alone could polarize B cells to a Breg phenotype. Polarization was attributed to high secretion of RANTES, MIP-α, TNF-α, and TGF-β; thus, at least some differentiation factors could be directly attributed to epithelial tumor cells.[57] Other signals, such as IL-21 and TGF-β, may be derived from other tumor-infiltrating or resident cells, as IL-21 is not known to be epithelial derived.[58]

Importantly, IL-10+ Bregs have been associated with tumor progression in experimental models[33,48–51,59–63] with some evidence to suggest their inhibitory role in human lung cancer (Fig. 1).[52,57] In murine preclinical models, data suggest that B cells may hamper chemotherapy. In an autochthonous transgenic murine model for prostate cancer, the presence of IgA/IL-10/PD-L1$^+$ plasma cells reduced tumor response to oxaliplatin, which could be restored in B cell-deficient mice.[59] Similarly, using a K14-HPV-induced squamous carcinoma model, Coussen's group has shown that cancer development was dependent on B cell-mediated FcγR engagement and that depletion of B cells (by anti-CD20) was complementary to platinum-based and Taxol-based chemotherapy for carcinoma.[33,60] Additionally, in murine models of lung metastasis, tumor angiogenesis is primarily dependent on B cell-mediated induction of VEGF, which requires Stat3 signaling in B cells.[61] Lung metastasis was shown to require the participation of active Stat3-expressing Bregs that can promote TGF-β-mediated conversion of CD4$^+$ TILs to Tregs, inhibit tumoricidal activity of NK cells, or directly activate MDSC.[62,63]

Data on the role of Bregs in human studies are more limited, with some indication of the presence of these cells in human lung cancer. For example, a high frequency of CD19$^+$IL-10$^+$ Bregs have been found within lung cancer patients,[57] and CD24^{hi}IL-10$^+$ B cells that display a plasma cell-like gene signature are elevated in lung tumors but not found in normal lung tissue.[52] Further, an increase in IL-10-producing Bregs is associated with an increased frequency of peripheral Tregs and MDSC in the advanced clinical stage of lung cancer,[64] suggesting that together with murine models, IL-10$^+$ Bregs may be detrimental to human disease. Breg activity has been found within Stat-regulated tumors, which together with NF-κB co-regulate numerous oncogenic and inflammatory genes.[65] Stat3-driven Bregs are negatively associated with survival in ovarian cancer[66] and have been identified in the tumor-draining lymph nodes (TDLN) of lung cancer patients.[67] These Bregs characterized by a CD19$^+$CD5$^+$ phenotype driven by Stat3 activation and IL-6-induced CD5 over-expression are thought to promote tumor progression by inducing tumor angiogenesis and immunosuppression through production of IL-10.[67] Together, the published data suggest that the presence of human Breg phenotypes maybe associated with inflammation; however, it is unclear whether they are bystanders or actively promote cancer.

Thus, the capacity of Bregs for attenuation of immune responses has been clearly demonstrated in multiple experimental models of cancer.[53] However, much remains to be understood regarding the biology of Bregs in human lung cancer. For example, the identification of a unique Breg signature, the mechanisms that control Breg differentiation, and a complete understanding of the interactions between Bregs and the tumor microenvironment will provide a clearer role of this population in lung cancer progression.

Table 2. Prognostic significance of TIB in human lung cancer

Markers		Methods	Studied cases	Tumor subtypes	Stages of the disease	Therapy	Location (mean extent) of infiltration	Follow-up (months)	Outcomes	Conclusion	Study
B cell antigen	BCL-6 CD21	IHC	91	ADC, SCC, LCC, bronchioloalveolar carcinoma, NSCLC-not specified	I to IV	Surgery	Intratumor or tumor margins	34	For all, stage, $p = 0.02011$	There was a strongly negative correlation between stage and the presence of intratumoral germinal centres.	Gottlin et al.[10]
	CD20	IHC	38	LCC only	I to III	Surgery	Intratumor	60	For all, OS, $p = 0.05$	A high number of intratumoral B cells had a significantly better survival than those with a low number of intratumoral B cells.	Eerola et al.[40]
	CD20	IF	202 (YTMA79)	ADC, SCC, other NSCLC	I to IV	ND	In the tumor or stromal tissue	60	For all, OS, HR = 0.523 (0.323–0.817), $p = 0.004$	High CD20 was statistically significantly associated with longer survival of NSCLC.	Schalper et al.[6]
	CD20	IHC	74	ADC, SCC	I or II	Surgery only	Tumoral stroma, invasive margin, or between tumor nests	60	For all, DSS, $p = 0.04$ OS, HR = 11 (1.4–9), $p = 0.02$	Low follicular B density correlated with poor survival of patients with NSCLC in both early-stage and advanced-stage.	Germain et al.[21]
			122	ADC, SCC, other NSCLC	III A or B	Surgery, chemotherapy, Others	Tumoral stroma, invasive margin, or between tumor nests	60	for all, DSS, $p = 0.06$ OS, HR = 2.1 (1.2–3.7), $p = 0.01$		
	CD20	IHC	218	ADC, non-ADC	I or III	Surgery, adjuvant treatment, chemotherapy, molecular targeted therapy, radiotherapy	ND	120	for all, OS, HR = 1.71, $p = 0.097$ RFS, HR = 1.96, $p = 0.004$ for ADC, OS, HR = 2.45, $p = 0.09$ RFS, HR = 2.86, $p < 0.01$ for ADC non-smokers, OS, HR = 0.22, $p = 0.03$ RFS, HR = 0.34, $p = 0.009$	A low accumulation of $CD20^+$ B cells was identified as an independent worse prognostic factor in patients with NSCLC, particularly in ADC and non-smokers ADC.	Kinoshita et al.[39]
	CD20	IHC	113	ADC, SCC, LCC, adenosquamous carcinomas	I to IV	Surgery	Peritumoral tissue	120	for all, OS, HR = 0.16 (0.06–0.42), $p = 0.04$ for non-SCC, OS, $p < 0.001$	The presence of B cells around the tumor margins was the only independent prognostic factor of NSCLC, and was relative to a significant survival advantage of non-SCC.	Pelletier et al.[68]

Table 2 continued

Markers	Methods	Studied cases	Tumor subtypes	Stages of the disease	Therapy	Location (mean extent) of infiltration	Follow-up (months)	Outcomes	Conclusion	Study
CD20	IHC	335	ADC, SCC, LCC, bronchioloalveolar carcinorma	I to IIIA	Surgery, postoperative radiotherapy	Intraepithelial tissue	60	for all, DSS, $p = 0.023$ for SCC, DSS, $p = 0.03$	Increasing numbers of epithelial $CD20^+$ and stromal $CD20^+$ lymphocytes correlated significantly with an improved DSS, limited to SCC.	Al-Shibli et al.[69]
						Tumoral stroma	60	for all, DSS, $p < 0.001$ for SCC, DSS, $p < 0.001$		
CD20 CD79	IHC	84 (training cohort)	ADC, SCC, LCC, adenosquamous carcinorma	I or II	Surgery only	Tumoral stroma	24	for all, RFS, HR = 1.99 (0.93–4.26), $p = 0.05$ RFS, HR = 1.47 (0.70–3.30), $p = 0.08$	A weak presence of $CD20^+$ B cells was confirmed in tumor microenvironment of the high-risk relapse subgroup of stage I/II NSCLC.	Hernandez-Prieto et al.[70]
CD20	IHC	111	ADC only	I to IIIA	Surgery only	Stroma	85	for all, DFS, $p < 0.001$ for stage I, DFS, $p < 0.001$	The infiltration of interfollicular B cells in cancer stroma was significantly associated with poorer prognosis when analyzed for all cases of lung ADC or for stage I cases alone.	Kurebayashi et al.[13]
CD20	IF	350 (YTMA140)	ADC, SCC, other NSCLC	I to IV	ND	In the tumor or stromal tissue	60	for all, OS, HR = 0.887 (0.643-1.236), $p = 0.447$	Increased CD20 signal was not associated with better survival of NSCLC.	Schalper et al.[6]
CD20	IHC	371	ADC, SCC, LCC	I to IIIA	Surgery, radiotherapy	Epithelial tissue	60	for all, DSS, $p = 0.059$	Both epithelial and stromal CD20 expression was not a prognostic factor for survival in NSCLC.	Hald et al.[71]
						Stromal tissue		for all, DSS, $p = 0.419$		
CD20	IHC	455	ADC only	I A or B	Surgery only	Tumor	60	for all, RFP, $p = 0.417$	Both tumoral and stromal $CD20^+$ cells had no significant prognostic value for patients with stage I lung ADC.	Suzuki et al.[72]
						Stroma		for all, RFP, $p = 0.389$		
CD20	IHC	56	SCLC only	I to IV	Surgery	Intratumor	60	for all, OS, $p = 0.634$	The number of intratumoral B cells was not significantly associated with survival.	Eerola et al.[73]

Table 2 continued

Markers		Methods	Studied cases	Tumor subtypes	Stages of the disease	Therapy	Location (mean extent) of infiltration	Follow-up (months)	Outcomes	Conclusion	Study
	CD138	IHC	355	ADC, SCC, LCC	I to IV	Surgery, adjuvant treatment	Tumoral stroma	58.7	for all, OS, HR = 0.74 (0.56–0.99), $p = 0.041$ for ADC, OS, HR = 0.54 (0.36–0.82), $p = 0.004$	Higher CD138 expression revealed a comparable association with longer survival of NSCLC, particular in the ADC subgroup.	Lohr et al.[35]
	CD138	IHC	371	ADC, SCC, LCC	I to IIIA	Surgery, radiotherapy	Epithelial tissue	60	for all, DSS, $p = 0.292$	Both epithelial and stromal CD138 expression was not a prognostic factor for survival in NSCLC.	Hald et al.[71]
							Stromal tissue		for all, DSS, $p = 0.165$		
	CD138	IHC	191	ADC, SCC, LCC	I to IIIA	Surgery, radiotherapy	Epithelial tissue	192	for all, DSS, $p = 0.847$	Both epithelial and stromal $CD138^+$ cells showed no significant correlation with DSS.	Al-Shibli et al.[77]
							Stromal tissue		for all, DSS, $p = 0.121$		
	p63 CD138	IHC	111	ADC only	I to IIIA	Surgery only	Stroma	85	for all, DFS, $p < 0.001$ for stage I, DFS, $p < 0.001$ for Papillary/Acinar Grade1/2 subtype, DFS, $p = 0.006$ for Papillary/Acinar Grade1/2 subtype (stage I) DFS, $p = 0.008$	The infiltration of interfollicular/parafollicular plasma cells in cancer stroma was significantly associated with poorer prognosis when analyzed for all cases of lung ADC or for stage I cases alone. The plasma cell infiltration is the independent negative prognostic factor in Grade1/2 papillary/acinar ADC.	Kurebayashi et al.[13]
	Immunoglobulin κC	IHC	355	ADC, SCC, LCC	I to IV	Surgery, adjuvant treatment	Tumoral stroma	58.7	for all, OS, HR = 0.72 (0.53–0.98), $p = 0.035$ for ADC, OS, HR = 0.57 (0.37–0.89), $p = 0.013$ RFS, $p = 0.044$	High immunoglobulin κC protein expression was associated with longer survival of NSCLC, particular in the ADC subgroup.	Lohr et al.[35]
	Immunoglobulin κC	qRT-PCR Microarray	196	ADC, SCC	I to IV	ND	Desmoplastic stroma in-between tumor cell nests	120	for all, OS, HR = 0.786 (0.722–0.856), $p < 0.001$ for ADC, OS, $p = 0.002$	Single immunoglobulin κC mRNA expression was significantly associated with longer survival of NSCLC. This prognostic relevance was only restricted to ADC.	Schmidt et al.[75]
			1056 (validation dataset)					ND	for all, OS, HR = 0.91 (0.85–0.98), $p = 0.011$		

Table 2 continued

Markers		Methods	Studied cases	Tumor subtypes	Stages of the disease	Therapy	Location (mean extent) of infiltration	Follow-up (months)	Outcomes	Conclusion	Study
	IgG_4	IHC	294	ADC, SCC, LCC, sarcomatoid carcinomas, adenosquamous carcinoma	I to IV	Surgery only	Stroma	60	for stage I SCC, OS, $p = 0.0409$ for SCC, OR = 15.4307 (4.5863–71.6024), $P < 0.0001$ for grade3 ADC, OR = 8.5662 (2.5946–38.8643), $P = 0.0013$	In patients with stage I SCC, IgG_4-producing plasma cells was significantly associated with a favorable prognosis.	Fujimoto et al.[76]
	$CD19^+CD20^+CD69^+ CD27^-CD21^-$	Flow cytometry	62	ADC, SCC and other	pT1a–pT4	Surgery, adjuvant treatment	ND	ND	ND	The survival of the small cohort of patients that had follow-up data were examined, which limited the assessment of predictive value of this Breg subset.	Bruno et al.[41]
	$CD19^+CD5^+ Stat3^+$	IF IHC	9	Lung and prostate cancer	ND	ND	ND	ND	ND	In lung tumor lymph nodes, p-STAT3 expression and CD5 positivity in $CD19^+$ B cells strictly correlated. However, the limited number of patient specimens failed to support the evidence that $CD19^+CD5^+$ B cells was a contributing negative factor for patient survival.	Zhang et al.[67]
B-cell signature	50-gene signature (mainly B/plasma cell immune genes)	Microarray	162 (validation dataset)	ADC, SCC, LCC, Adenosquamous	I or II	Surgery only	Tumoral stroma	24	for all, RFS, HR = 3.44 (1.6–7.3), $p = 0.001$	The predictor 50-gene was over-expressed in the low- compared to the high-risk relapse group of stage I/II NSCLC.	Hernandez-Prieto et al.[70]
	60-gene signature (primarily humoral immunity related genes)	qRT-PCR Microarray	196	ADC, SCC	I to IV	ND	Desmoplastic stroma in-between tumor cell nests	120	for all, OS, HR = 0.786 (0.722–0.856), $p < 0.001$ for ADC, OS, $p = 0.002$	The 60-gene signature was significantly associated with longer survival of NSCLC. This prognostic relevance was only restricted to ADC.	Schmidt et al.[75]
	60-gene signature (primarily humoral immunity related genes)	mRNA-seq	504	ADC, SCC	I to IV	ND	ND	ND	for ADC, OS, HR = 0.71 (0.58–0.87), $p = 7.80E-04$	High expression of 60-gene signature predicted improved overall survival of ADC.	Iglesia et al.[83]
	24-gene signature (mainly B cell lineage-related genes)	Microarray	130	SCC only	I to III	Surgery, adjuvant treatment	ND	72	for stage I–II SCC, OS, $p < 0.001$	B cell-related genes dominated the list of 24-gene signature in early stage SCC of the lung.	Mount et al.[84]

Table 2 continued

Markers		Methods	Studied cases	Tumor subtypes	Stages of the disease	Therapy	Location (mean extent) of infiltration	Follow-up (months)	Outcomes	Conclusion	Study
	B cell specific signatures	mRNA-seq or Microarray	933	ADC, SCC	I to IV	ND	ND	ND	for ADC, OS, HR = 0.870 (0.719–1.053), $p = 0.154$ for bronchioid, OS, HR = 0.908 (0.616–1.337), $p = 0.625$ for magnoid, OS, HR = 0.930 (0.669–1.293), $p = 0.666$ for squamoid, OS, HR = 0.747 (0.549–1.015), $p = 0.062$ for SCC, OS, HR = 1.001 (0.831–1.206), $p = 0.992$ for basal, OS, HR = 1.038 (0.726–1.483), $p = 0.840$ for classical, OS, HR = 1.155 (0.832–1.603), $p = 0.390$ for secretory, OS, HR = 0.977 (0.690–1.384), $p = 0.896$ for primitive, OS, HR = 0.667 (0.330–1.349), $p = 0.260$	B cell infiltration was associated with relatively better prognosis of squamoid subtype, but can not predict survival of four SCC subtypes.	Faruki et al.[74]
	B cell specific signatures	mRNA-seq or Microarray	1336	ADC, SCC, LCC, SCLC	I to IV	Surgery only	Intratumor	185	for ADC, Naive B, outcome, z-score = −1.43, $p > 0.05$ Memory B, outcome, z-score = −1.40, $p > 0.05$ Plasma cell, outcome, z-score = −1.59, $p > 0.05$ for SCC, Memory B, outcome, z-score = 2.02, $p < 0.04$ for LCC, Plasma cell, outcome, z-score = 2.71, $p < 0.04$	The three subgroups of B cell representation including naive, memory and plasma cells have a strong trend of favorable outcomes of lung ADC, whereas memory B cell and plasma cell signatures denoted adverse outcomes of SCC and LCC, respectively.	Gentles et al.[82]

HR: hazard ratio, *OR:* odd ratio, *ND:* Not Determined.

PREDICTIVE VALUE OF TIB FOR THE PROGNOSIS OF HUMAN LUNG CANCER

The presence of B cells in human lung tumors has been correlated with patient prognosis, as summarized in Table 2. The number of intratumoral GCs and/or B cells was found to be inversely correlated with lung cancer stage.[10] Generally, when identified by CD20 for total B cells, the presence of B cells infiltrating NSCLC tumor is overwhelmingly favorable for long-term overall survival (OS), recurrence-free survival (RFS), or disease-specific survival (DSS) of NSCLC,[6,21, 39,40,68–70] with a few exceptions that did not identify any significant associations[6,71,72] or negative associations with the disease.[13] No significant association between TIBs and prognosis was observed for small cell lung cancer (SCLC).[73] Among studies that show favorable TIB presence, no difference in prognostic outcome was observed between patients with early-stage or late-stage tumors,[21] suggesting that the presence of B cells can affect outcome at all stages of disease. Further, the presence of common driver mutations such as EGFR, KRAS, and TP53 in lung adenocarcinoma (ADC) was found to have no effect on B cell infiltration or their prognostic values.[52,74]

Although most studies indicate a favorable outcome of TIBs in NSCLC, prognostic impact differs when analyzed with clinical correlates such as histological subtype and location. In studies with mixed tumor subtypes, most studies indicate that the prognostic relevance was restricted to ADC,[39,68] with one study showing relevance in only SCC[69] or LCC.[40] Further, when the location of TIBs was analyzed for all NSCLC subtypes, most studies indicated that a stromal[6,21,70] or marginal[68] infiltrate, rather than an intraepithelial infiltrate, was associated with favorable prognosis. Interestingly, a favorable outcome for intraepithelial TIBs was only observed in samples that show SCC or LCC as the prognostic subtype,[40,69] suggesting that mechanisms of B cell regulation of tumors may differ among lung cancer subtypes. The prognostic difference observed among studies may be due to the interpatient heterogeneity within the study cohorts or biological differences in TIB infiltration among the tumor subtypes. For example, a greater abundance of $CD20^+$ B cell infiltration has been observed in ADC than in SCC or LCC.[6,40] Interestingly, SCLC has a greater abundance of infiltrating T cells and TAMs but few TIBs, which may account for the low prognostic value of TIBs in SCLC.[73]

In addition to the beneficial prognostic value of total B cell infiltration, the role of tumor-infiltrating plasma cells, identified as $CD138^+$ cells, has been explored. Unlike the overwhelming evidence for the prognostic benefit of total B cell infiltration, plasma cell analysis has yielded more conflicting results, with data correlating to favorable prognosis,[35,75,76] no prognostic value,[71,77] or adverse prognosis.[13] Analysis of clinical correlates such as NSCLC subtype or location could not explain the differences observed among different studies, as both ADC or SCC subtypes with stromal infiltrating plasma cells were shown to have favorable outcomes in some reports[35,75,76] but not by others when similar subtypes and locations were analyzed.[71,77] One ADC study deviates from the others that indicate a favorable role of B cells in prognosis. Kurebayashi et al. show that when analyzed in the presence of other immune cells, high infiltrates of plasma cells may be indicative of poor outcomes, suggesting that the prognostic value of B cells may change in the context of other cells.[13] Notably, four studies have performed additional analysis of immunoglobulin isotypes.[13,35,75,76] IgG_4-producing plasma cells were determined to be a positive prognostic factor in SCC.[76] In contrast, subset analysis of an ADC cohort demonstrated that the presence of IgA^+ rather than IgG_4^+ plasma cells trended towards an adverse prognosis; however, statistical significance was not reached.[13] This observation is consistent with the known immunosuppressive effects of IgA^+ plasma cells on chemotherapy models,[59] as well as with IgG-positive cells ($IG\kappa C^+$ cells) as good predictors in ADC.[35,75] Overall, given that class-switching and the role of immunoglobulins is considerably affected by different cytokine/chemokine milieus, such as Th2-biased conditions to support regulatory IgG_4 or IgA production by plasma cells in the human melanoma microenvironment,[78] differences in the presence or effects of each immunoglobulin may exist between each histological subtype of lung cancer.[79] Further studies of isotype-specific plasma cells in different subtypes of lung cancer are required to determine this relationship.

Molecular characterization of NSCLC has revolutionized our understanding of lung cancer.[80,81] Coupled with immune subset deconvolution algorithms,[82] a more complete understanding of the role of immune infiltrates can be determined with the genomic data alone. Among genomic studies, several B cell-associated gene signatures have been found to correlate with lung cancer prognosis. Gene signatures consisting primarily of humoral immunity-related genes have been shown to predict improved OS in lung ADC.[70,75,83] In addition, a 24-gene signature, dominated by B cell lineage-related genes, was identified to be predictive of clinical outcome of early-stage SCC.[84] Finally, in a pan-cancer analysis of leukocyte gene signatures, analysis of B cell subgroup signatures, including naive, memory B cells and plasma cells, showed that all three B cell signatures have a strong trend of favorable outcomes of lung ADC, whereas memory B cell and plasma cell signatures significantly denoted adverse outcomes of SCC and LCC, respectively.[82] Overall, similar to the histological identification of B cells, the genomic presence of B cells, including pan-B cells and B cell subtypes, generally shows different prognosis outcomes among subtypes.

Beyond standard histological classification of tumor subtypes, molecular characterization allows for additional stratification of NSCLC ADC or SCC. Differences among molecular subtypes and B cell signatures have been observed.[74] In analyses considering the molecular subtypes of ADC or SCC, immune infiltrates have been associated with survival in NSCLC. For example, B cell signature is higher in the “squamoid” and “bronchioid” molecular subtypes of lung ADC, while the presence of TIBs is associated with relatively better prognosis of only the squamoid subtype.[74] The analyses of the representation of the B cell subgroup and molecular subtype of lung cancer can optimize and improve the assessment of the prognostic impact of TIBs in lung cancer.

The lack of a universally accepted method for evaluating antitumor versus protumor B cells makes it difficult to evaluate the role of TIBs in cancer. In general, the presence of $CD20^+$ B cells or IgG^+ plasma cells identified by IHC or gene signature analysis indicates their presence to be beneficial. On the other hand, a few histological studies have demonstrated a detrimental role of B cells on prognosis and are possibly limited due to technical restraints. For example, the use of a single antigen, $CD20^+$, would not differentiate between antitumor B cells and Bregs or between activated and exhausted B cells in TIBs.[41] A similar problem can be observed in studies analyzing only CD138 expression. $CD138^+$ B cells only represent a part of plasma cells[82] and thus may underestimate the presence of anti-tumor antibody-generating cells or potentially overestimate plasma cells that generate non-tumor-recognizing antibodies, leading to the conclusion that $CD138^+$ cells have no prognostic relevance in some studies.[71,77] As discussed above, studies have described $CD19^+CD20^+CD69^+CD27^-CD21^-$ and $CD19^+CD5^+$ B cells as the pathogenic populations within lung tumors,[41,67] but as Bregs share common markers with anti-tumor B cells, their contribution will be difficult to discern. Future research efforts should focus on investigating biomarkers that might differentiate anti-tumor B cells from Bregs and developing more robust analytical platforms that combine multiple techniques such as multi-chromatic flow cytometry, IHC, and/or gene sequencing studies together with data from clinical outcomes.

Table 3. B cell-based immunotherapeutic strategies for lung cancer

Therapeutic method	Phase/condition	Experimental arm (s)	Control arm(s)	Mechanism	Efficiency	Study
Adoptive transfer B cells	B16-F10 pulmonary metastatic C57BL/6J mice model	CpG-activated B cells	Spleen-derived $CD19^+$ B cells only or CpG only or no treatment	Reducing immunosuppressive environment by lowing recruitment of Tregs and MDSCs and decreasing release of IL-10 and TGF-β	Adoptive transfer of CpG-treated B cells facilitates tumor regression and reduces lung metastasis.	Sorrentino et al.[86]
	MCA 205 or D5 pulmonary metastatic C57BL/6 mice model	MCA 205 or D5G6 TDLN-B cells[a]	MCA 205 or D5G6 TDLN-B[a] + T[b] cells or TDLN-T cells[b] only or no treatment	Strong humoral responses to tumor, which displays that immunoglobulins specifically recognize and lyse tumor cells in the presence of complement	The activated TDLN-B cells[a] mediate significantly greater reduction of tumor lung metastases upon adoptive transfer.	Li et al.[87]
	4T1 pulmonary metastatic BALB/c mice model	$IL\text{-}10^{-/-}$ 4T1 TDLN-B cells[a]	WT 4T1 TDLN-B cells[a] or IL-2 only or no treatment	Directing tumoricidal-cell response via the Fas/FasL pathway	Adoptively transferred B cells effectively reduce number of pulmonary metastatic nodules.	Tao et al.[88]
		IL-10 antibody + WT 4T1 TDLN-B cells[a]	WT 4T1 TDLN-B cells[a] or WT 4T1 TDLN-B cells[a] + IgG_1 or IL-10 antibody only or no treatment			
	4T1.2 pulmonary metastatic BALB/c mice model	CXCL13-coupled CpG-ODN	CpG-control or mock	Rendering B cells stimulatory to induce cytolytic $CD8^+$ T cell responses	In vivo-targeted delivery of CpG-ODN to B cells blocks lung metastasis.	Bodogai et al.[89]
In vivo sensitized B cells	Lewis lung carcinoma C57BL/6J mice model	FMS	DFMS or no treatment	Increasing antibody-mediated cytotoxicity involving the production of IgM against tumor-specific glycans by $CD138^+$ B cells	Immunization of FMS stimulates anti-tumor activities of B cells to suppress lung tumor growth.	Liao et al.[90]
	76-9 rhabdomyosarcoma pulmonary metastatic C57BL/6J mice model	B cell-deficient mice or B cell-deficient mice + CY or B cell-deficient mice + CY + IL-15	C57BL/6J mice with NK cell impaired, both T and B cell-deficient, T cell-deficient, mutants deficient in αβ-T cells, γδ-T cells, both αβ-and γδ-T cells or these mice + CY or these mice + CY + IL-15	Antagonizing NK and T cell-mediated effects	B cell-deficient mice with 76-9 tumor lung metastases show a significant improvement in the survival rate.	Chapoval et al.[91]
B cell depletion	TC1 and LKR C57BL/6J mice model	Anti-CD20 antibody (18B12)	Ad.E7 or combination 18B12 with Ad.E7 or no treatment	Increasing the number and activity of systemic and local tumor $CD8^+$ T cells	B cell depletion using anti-CD20 retards lung tumor growth. B cell depletion in conjunction with an adenovirus vaccine markedly augments anti-tumor efficacy, resulting in lung tumor regression	Kim et al.[92]
	4T1.2 pulmonary metastatic BALB/c mice model	Anti-CD20 antibody (5D2) /Rituximab	IgG control or mock	Enriching for $CD20^{low}$ Bregs, which mediates immunosuppression	Anti-CD20/rituximab enhances tumor progression and lung metastasis.	Bodogai et al.[89]
	4T1.2 pulmonary metastatic BALB/c mice model	CXCL13-coupled CpG-ODN	CpG-control or mock	Inactivating Bregs by reversing the expression of CD137L	In vivo-targeted delivery of CpG-ODN to B cells blocks lung metastasis.	Bodogai et al.[89]
Breg inactivation	4T1 pulmonary metastatic BALB/c mice model	Resveratrol	Mock	Abrogating Breg generation and Breg-mediated Treg conversion through the inactivation of Stat3	Resveratrol-induced inactivation of Bregs is sufficient to inhibit lung metastasis.	Lee-Chang et al.[94]
	4T1 pulmonary metastatic BALB/c mice model	MK886	Mock	Inactivating the 5-lipoxygenase/leukotriene/PPARα pathways in Bregs	MK886-triggered inhibition of Btrgs abrogates cancer escape and metastasis.	Wejksza et al.[97]

Experimental models of tumor pulmonary metastasis have been used as substitutes to study the lung cancer immune microenvironment and antitumor immune mechanism by B cells in orthotopic lung cancer[86,91]

FMS: a l-fucose (Fuc)-enriched Reishi polysaccharide fraction, *Ad.E7:* adenoviral tumor antigen vaccine

[a]Activated in vitro with LPS/anti-CD40

[b]Activated in vitro with anti-CD3/anti-CD28/IL-2

TIB-BASED IMMUNOTHERAPY IN LUNG CANCER

Despite advances in surgery, chemotherapy and radiation therapy, lung cancer continues to have a low survival rate.[1,85] Given the prognostic benefit of B cells, development of B cell-based immunotherapeutic strategies may be attractive.[14] Targeted approaches for enhancing conventional B cells through stimulation with B cell ligands can effectively inhibit tumor growth and lung metastasis, as exhibited in several murine models (Table 3). After activation in vitro, CpG-activated B cells and TDLN-B cells have been shown to display immunoenhancing properties after adoptive transfer.[86–88] Therapeutic efficacies of these B cells were associated with increased antitumor responses by antibody-specific recognition, lysis of tumor cells, reduction of the immunosuppressive environment, and/or tumoricidal-cell response via the Fas/FasL pathway. Additionally, B cells that are activated directly in vivo show effective tumor inhibition. For example, CXCR5-expressing B cells, stimulated by CXCL13-coupled CpG-ODN, can trigger the cytolytic effect of $CD8^+$ TILs, leading to abrogation of lung metastases in 4T1.2 tumor-bearing mice.[89] Further, natural compounds, such as Reishi polysaccharide fraction, can suppress tumor growth using $CD138^+$ B cells via activating IgM-mediated cytotoxicity, as exhibited in a Lewis lung tumor mouse model.[90]

Alternatively, given the role of Bregs, strategies have been devised in experimental models that target depletion/reversal of pathological B cells (Table 3). Early tumor pulmonary metastasis models showed significant improvement in survival by depleting B cells with anti-CD20 antibodies, including rituximab.[91,92] Unfortunately, there was no marked clinical benefit in some solid tumors, such as renal cell, colorectal carcinoma, and melanoma.[14] Because $CD20^+$ B cells have been shown to correlate with good outcomes in NSCLC,[21] depletion of $CD20^+$ B cells could be detrimental to lung cancer treatment.[93] Indeed, it was subsequently found that anti-CD20 antibodies may deplete antitumor B cells and possibly enrich for $CD20^{low}$ Bregs, thus exacerbating tumor growth.[89] Recently, several chemical modulators have been observed to selectively inhibit Bregs. For example, CpG-ODN significantly reduced $CD20^{low}$ Bregs while activating antitumor B cells, leading to inhibition of lung metastasis in 4T1 tumor-bearing mice. Resveratrol efficiently prevented the generation and function of Bregs in a similar animal model.[94] Other mediators, such as total glucosides of paeony and Lipoxin A4, also exerted an antitumor effect by inhibiting Bregs.[95,96] These molecules attenuated the expansion of Bregs by inactivating Stat3 and/or ERK, leading to a reduction in IL-10 or TGF-β, which further decreases Breg-induced Treg generation.[94,96] In addition, the inactivation of the 5-lipoxygenase/leukotriene/PPARα pathways in Bregs, for example, using MK886 is sufficient to abrogate cancer escape and metastasis through the loss of Tregs and the release of effector $CD8^+$ T cells in lung metastasis.[97] Overall, B cell-based therapies, except rituximab, are still in preclinical studies, which are classified into three major strategies, including adoptive transfer of stimulated effector B cells, antitumor B cell activation and Breg inhibition in vivo. These B cell-based strategies primarily promote cytotoxic T cells and/or inhibit downstream immunosuppressive pathways, resulting in antitumor immune responses. Future studies will be needed to develop approaches to effectively induce B cells to amplify antitumor responses by other immune cells.

CONCLUSION AND PERSPECTIVE

Our current understanding indicates that tumor-infiltrating B cells are important regulators of lung cancer progression. Driven by signals within the tumor microenvironment, B cells infiltrate, proliferate, and develop in tumors. TIB exert anti-tumor immunity through secretion of tumor-specific antibodies, promoting T cell responses, and maintaining the structure and function of TLS, all of which are associated with beneficial outcomes for lung cancer. However, as multifaceted effectors, B cells can develop into IL-10-secreting immunosuppressive phenotypes, leading to tumor progression. Novel immunotherapeutic strategies targeting B cells will have to both promote antitumor B cells and inhibit Breg phenotypes. Further exploration of B cell function within the lung tumor microenvironment will allow for improved therapeutic strategies to target this important subset of immune cells.

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ADDITIONAL INFORMATION

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