B Cells and Tertiary Lymphoid Structures: Friends or Foes in Cancer Immunotherapy?

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ABSTRACT

Tumor cells pose a challenge to the adaptive immune system, and its key cell types, T and B cells, have frequently been associated with an improved prognosis. The success of immune checkpoint blockade has confirmed the relevance of T cells. However, the role of B cells is increasingly recognized, and highlighted in this review. Recent data suggest that tumors contain a diverse set of B cells reflecting different developmental states and exerting functions such as antigen presentation, antibody production, and regulatory effects. Further, B cells are frequently located in tertiary lymphoid structures (TLS), which are immune cell niches that sustain an immune response at sites of chronic inflammation. TLSs in tumors display substantial heterogeneity, ranging from cell aggregates to mature structures with an active germinal center. Recent studies have provided insights into initiation, cellular and spatial composition, and function of TLS in a variety of cancer types; however, several critical issues still need to be resolved. Currently, initial reports are discerning the role of TLSs in immunotherapy, with the majority of studies observing TLSs to confer favorable patient outcome. Finally, TLS induction in tumors is evaluated, with the therapeutic aim to reactivate the host immune response.

Introduction

In malignant tumors, cancer cells actively crosstalk with the host immune system and the surrounding stroma cells to promote continuous growth. During the last decade, it has become exceedingly clear that targeting or taking advantage of the immune system is a highly feasible therapeutic option for many cancers. In particular, reactivating tumor-specific T cells by blocking immune checkpoint molecules, using PD-1 and CTLA4-blocking antibodies has opened a new avenue of treating patients with cancer. The search for predictive biomarkers to immune checkpoint blockade (ICB) within the scientific community is extensive. One of the most promising biomarkers associated with improved clinical response to ICB is the presence of B cells and tertiary lymphoid structures (TLS) in pretreatment tumor tissue. However, the exact mechanism on how TLSs improve T-cell fitness, if antibody-producing B cells are essential, and which cells are crucial for these processes, is still unknown.

In this review, we first discuss normal B-cell development and explore the different B cell types existing in tumors and TLSs. We then elaborate on the findings on how tumor-associated TLS are formed and differentiated. In addition, a section on the immunosuppressive functions of regulatory B cells (Breg) is included. Finally, we focus on the clinical implications of TLSs in tumors, especially in the light of several seminal studies that identified a predictive role of TLSs to ICB. This review summarizes the current understanding of the underlying mechanisms of TLS formation in tumors. The recent studies unraveling the impact of tumor-associated B cells and TLSs support a crucial role in cancer immunology.

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The Role of B Cells in Tumors

B-cell development

Human B cells derive from the common lymphocyte progenitor lineage and split in B-1 and B-2 cells. B-1 cells have limited B cell receptors (BCR) specificity and are associated with innate immune system processes. B-2 cells, which are the conventional B cells, migrate from the bone marrow to the spleen to differentiate to marginal B cells that remain in the spleen and follicular B cells that move on to populate the lymph nodes. B cells pass the pro-B and pre-B-cell stages to become immature/naïve B cells. Thereby, the heavy chain undergoes recombination and, together with a surrogate light chain forms the pre-BCR in pre-B cells. Then, the light chain by recombining the kappa light chain, or in the case of autoreactivity recombination of the lambda light chain, completes the BCR (1). Upon contact with their cognate antigen, B cells further undergo affinity maturation and class switch recombination (CSR) and can develop into either memory B cells or antibody-producing plasma cells. Affinity maturation occurs in germinal centers of lymph node follicles and involves somatic hypermutation (SHM) of the BCR sequence to increase antigen specificity. The gene *AICDA* that drives SHM also induces CSR, by which the BCR isotype is changed from IgD/M to IgG/A/E. IFNγ promotes the IgG, and TGFβ promotes the IgA isotype (1). Antibodies exert antitumor functions by activation of the complement system; natural killer cells and macrophages can bind to antibodies via Fc receptors to attack tumor cells, and dendritic cells can use antibodies to internalize antigens for presentation. In addition, B cells can also capture and internalize antigens with their BCR to present the antigens to CD4 (via MHC-II) and CD8 cells (via MHC-I). Overall, the functional repertoire of B cells is thought to consist of antibody production, presentation of antigens, and the release of cytokines and cytotoxic effector molecules.

B-cell states and functions in cancer

B cells in the tumor microenvironment (TME) span many B-cell states, with particular rich insights coming from single-cell RNA sequencing (scRNAseq) efforts. In melanoma, B cells were divided

into four groups: one unswitched (IgD+), two switched (IgD-), and one plasma cell group. Protein analyses, including CD27/CD38/IgD markers, could confirm these B-cell groups (2). B cells from the Jerby-Arnon scRNAseq dataset (3) segregated by the expression of surrogate light chain *IGLL5* and activation marker *CD69* genes, while at the same time, early B-cell stage genes were abundantly expressed (4). In both melanoma datasets, plasma cells constituted only a small fraction of the B cells. In breast cancer, Hu and colleagues observed naive B cells, memory B cells, and smaller clusters of plasma cells and germinal center cells. B cells mostly expressed IgM and IgG. Interestingly, tumor-associated B cells had higher levels of SHM and were more clonally expanded than B cells from peripheral blood. Memory B cells as well as plasma cells could share VDJ sequences, that is, having clonal origin, and plasma cells in addition could share SHM sequences, that is, being clonal (5). In a mouse model of triple-negative breast cancer, scRNAseq identified one large unswitched B-cell group, a small switched AICDA+ group, and one small plasma cell group (6). Finally, in lung adenocarcinoma, scRNAseq data revealed a small germinal center B-cell group, with expression of *AICDA, BCL6*, and *CD86*, as well as a follicular B-cell group. Interestingly, a CD4 T-cell group resembled T follicular helper (Tfh) cells expressing *CXCR5, BCL6*, and *PD-1* (7).

In human papillomavirus (HPV)-positive head and neck cancer, the scRNAseq data of B cells converged on plasma cells (*XBP1*+, *PRDM1*+, *SDC1*+, *CD20*−), germinal center cells, and activated cells which did not display prominent markers. In this regard, HPV viral antigens may recruit different B-cell sets as in neoantigen-based tumor immunity (8). Another specialized function of B cells has been observed in ovarian cancer, where B cells and plasma cells preferentially express IgA, which is directed at antigens but also is internalized by tumor cells via PIGR in an antigen-independent fashion. This internalization sensitizes the tumors for T-cell attack (9). In bulk tumors, a comprehensive study investigated the hypervariable CDR3 sequences and BCR isotypes in a pan-cancer analysis (10). They found BCR sequences to overall consist of 60% IgG, 35% IgA, and 4% IgM isotypes. Interestingly, hardly any IgD was found which is in contrast to scRNAseq data. IgG isotypes presented with elevated SHM levels. Clusters of clonally related BCRs often shared different IgG isotypes, particularly between G1 and G3 isotypes, suggesting a shift between G1 and G3 isotypes during immune response, whereby the G1 isotype potentially triggers a more cytotoxic response (11). Isotype sharing between IgA and IgG was found to be rare (10). Tumor antigens targeted by antibodies consist of mutated and wild-type amino acid sequences. Humoral responses against a number of antigens, such as mutated p53, have been described (12). Further, antibodies derived from immortalized ovarian cancer B cells were found to bind to many secreted and extracellular domain-containing proteins (9). However, a comprehensive catalog of antigens targeted by B cells in the tumor tissue is still needed.

In summary, although tumor-associated B-cell states are cancer type–dependent, frequently an unswitched naïve-like state and a switched state with more or less memory-like phenotype are observed. In addition, rare populations of proliferating germinal center cells, as well as plasma cells have been identified. Also, the understanding of isotype distribution and clonal relationship of B cells is increasing. However, the exact functional role of these relatively roughly described B-cell states in the TME and which antigens these B cells are reactive to, is still elusive.

Moreover, several studies also describe Bregs that secrete IL10, IL35, and TGFβ, tend to have BCRs of the IgA isotype, and exert immunosuppressive functions. Bregs were not explicitly observed in scRNA-seq data, but have instead been identified by protein analyses using surface makers. However, in contrast to the defining *FOXP3* expression by regulatory T cells (Treg), Bregs do not display a uniform surface marker or transcription factor. The surface markers that have been used range from immature markers, such as CD1D and CD5, to markers of effector stages, such as CD27 (13). Consequently, a variety of Breg subsets have been reported and are thought to emerge from various stages of B-cell development (14). The Breg subsets between mice and human tumors are likely also differing. In human tumors, Bregs have been observed in several cancer types, including gastric cancer, hepatocellular carcinoma, head and neck cancer, pancreatic cancer, breast cancer, and colon cancer (13, 14). To support an immunosuppressive TME, Bregs have been suggested to induce Treg polarization, inhibit T-cell effector functions and cross-talk with other immune cells (13), yet the precise interplay in the TME remains to be resolved. Also, it is not clear whether Bregs are localized in immune niches within the tumor or are more scattered throughout the tumor parenchyma. Bregs have been associated with a poor prognosis (15, 16), which is in contrast to the general positive role of B cells in the majority of cancer types. In addition, circulating Bregs in peripheral blood have been linked to poor outcome in patients treated with ICB (17). Together, Bregs have been described in several tumor types, however, their role needs to be further specified.

How TLSs Are Developed in Tumors

Inducers, organizers, and chemokines

TLSs are ectopic aggregates of immune cells with similarities to secondary lymphoid organs (SLO). The presence of a B-cell zone and T-cell zone is required for TLS definition. However, other cell types such as dendritic cells, high endothelial venules (HEV), or fibroblasts can also be accrued to the TLS (**Fig. 1**; ref. 18). TLSs have been reported in chronic inflammation, autoimmune disease, organ transplantation, and, importantly in cancer, and are supposed to act as niches for immune processes such as antigen presentation and antibody production. SLOs develop at predetermined sites by the interaction of a lymphoid tissue inducer cell (LTi) with a lymphoid tissue organizer cell (LTo). In lymph nodes, the LTi cell is an innate lymphoid cell 3. The LTo cell is a mesenchymal cell that later differentiates into follicular dendritic cells (FDC) and fibroblastic reticular cells (FRC). For TLS located in tumors these cells are surrogated, and the exact cell of origin is not clearly resolved in humans. In a mouse tumor model that presents with TLS, the surrogate LTi were found to be T cells and B cells, both expressing *LTA*, whereas the surrogate LTo were $PDPN^+$ fibroblasts expressing the receptor *LTBR* (19). The expression of the key chemokines CXCL13, and CCL19/CCL21 further attracts CXCR5 (receptor to CXCL13) and CCR7 (receptor to CCL19 and CCL21) positive cells to the site (20). CXCR5-expressing cells are composed of B cells and T follicular helper (Tfh) cells, but of note, a subset of Tfh cells has been reported to be CXCR5 negative (21). CCR7 is expressed on a wider range of T-cell subsets, yet of a more naïve-like phenotype (22). CXCL13 is expressed by FDCs in SLOs, whereas this role is likely taken by T cells in TLS (23, 24). CCL19 and CCL21 are expressed by FRC in SLOs, which presumably are surrogated in tumor TLS, whereby CCL21 is thought to stem from endothelial cells (25). For instance, in lung cancer, CCL19 was predominantly expressed by the $LAMP3^+$ dendritic cells and CCL21 was restricted to $PDPN^+$ lymphatic vessels (26). It is however possible that different surrogate cells are used by the TME of various cancer types. In addition, HEV within TLSs express peripheral node addressin (PNAd) that home in immune cells expressing the receptor *SELL* (CD62L; ref. 27). Furthermore,

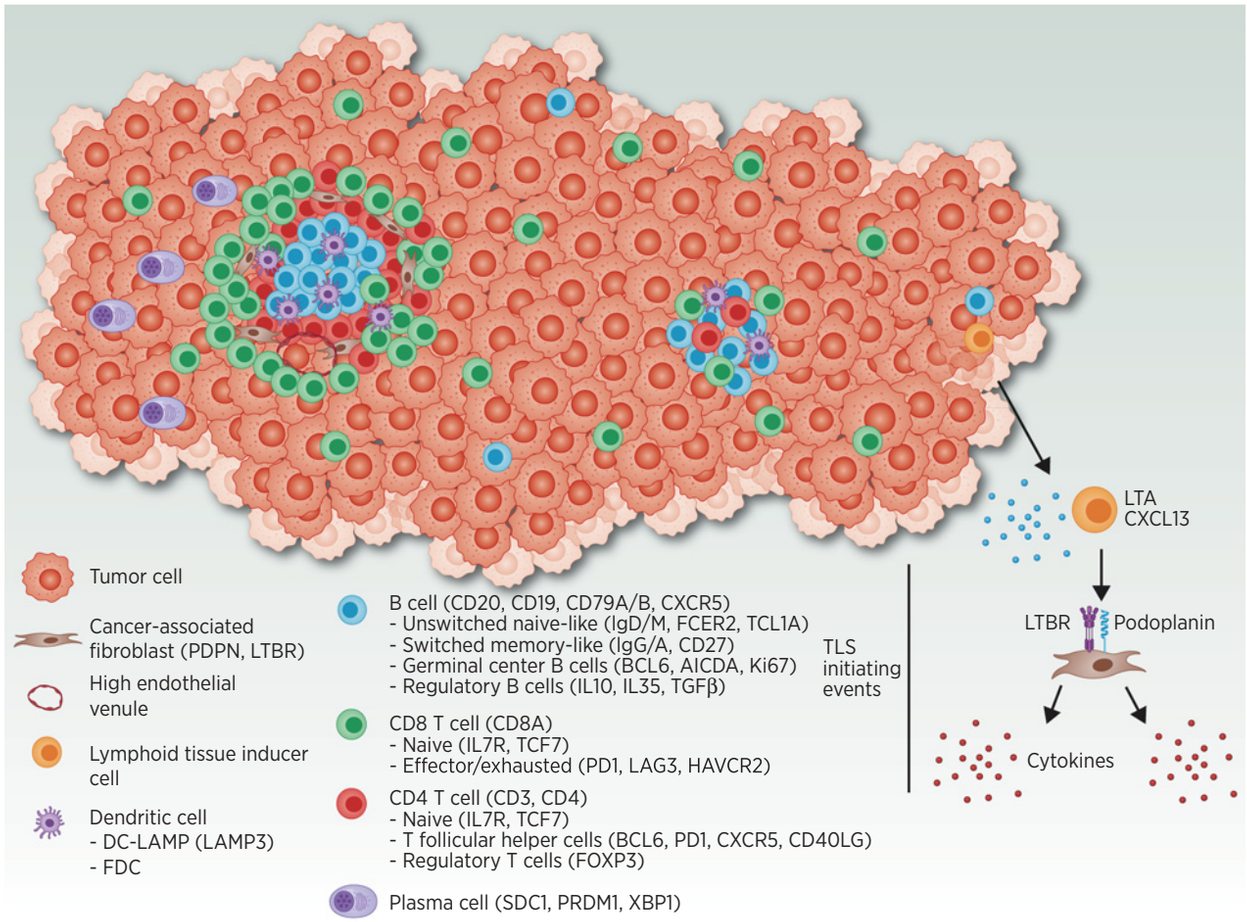

Figure 1.
Model of TLS development. TLSs span a gradient of complexity, from more immature cell aggregates to mature structures with a germinal center. Several subsets of B and T cells have been observed; their functional role, recruitment, and spatial distribution is currently investigated. DC-LAMP = DC-LAMP+ dendritic cell. Adapted from an image created with BioRender.com.

activated fibroblasts create a chemokine niche where antigen presentation between immune cells is enabled. This niche is further reenforced by *CXCL13* expression (25). Together, a specific set of cells and chemokines seems to orchestrate TLS formation in cancer; however, the detailed mechanism and variation depending on the TME context is still to be resolved.

Germinal center

In lymph nodes, a primary follicle consists of unswitched naïve B cells, which upon antigen exposure can turn into a secondary follicle with an oligoclonal germinal center. The germinal center consists of a dark zone (DZ) and a light zone (LZ). The B cells cycle between the zones along the chemokine gradient of CXCL12 and CXCL13 using the respective receptors CXCR4 and CXCR5. In the DZ, the B cells proliferate and undergo SHM, whereas in the LZ, the B cells internalize high affinity antigens and compete for stimulus from Tfh cells via CD40 and ICOSLG. Repeating these cycles, B cells with BCRs of increased antigen affinity are selected, which can further turn into memory B cells or antibody-producing plasma cells (28). In tumor TLS, germinal centers and antibody production are also observed (29), however at varying frequencies (4, 30). The master gene of GC initiation and DZ orchestration *BCL6*, as well as AID/*AICDA* (SHM and CSR) and KI67 (GC proliferation) were also reported to be expressed in TLS (31).

Heterogeneity of TLSs

TLSs have been observed in a large number of cancer types so far (32–37), even in immune poor cancers such as glioma (38). Yet, TLSs are remarkably heterogeneous, within a cancer type as well as across the cancer spectrum. Numerically, TLS abundance can vary considerably between early and late disease stage depending on tumor type (37). For metastatic sites, TLS densities also depend on tumor type and can even be absent, for example, TLS were not detected in brain metastases in a breast cancer study (39). TLS at both primary and metastatic sites tend to confer good outcomes to patients. However, heterogeneity of TLS composition at primary and various metastatic sites, also within the same patient, clearly requires more investigations. In melanoma, TLSs have been found in varying degrees of maturation (40), with presence of a germinal center being considered as the most mature type of TLS (**Fig. 1**). As B cells are consistently surrounded by T cells in melanoma, yet, morphology, maturity level, and GC formation of TLSs is highly varying (4). Remarkably, melanoma tumors with germinal center–like TLSs coexisted with TLSs of a more

immature morphology (4). It has been suggested that TLSs lacking a mature GC may be described by the term "lymphoid aggregate"; however, this concept is undermined by a wider gradient of TLS heterogeneity observed in tumors. In a sarcoma study, due to pronounced heterogeneity, TLSs were divided into early, primary-, and secondary follicle–like TLS, of which 61% were early TLS (30). This subdivision was initially proposed for TLSs from colorectal cancer (41) and was also applied in urothelial cancers (42). A recent study suggested that an additional immune cell niche exist in tumors. $TCF7^+$ CD8 T cells colocalized with the antigen presentation complex MHC-II in certain regions, which resembled more T-cell zones of lymph nodes rather than TLSs (43). Together, TLSs consist of a gradient from rather loose B- and T-cell aggregates to complex structures with a mature germinal center.

Cellular composition of TLS

TLS have been called from hematoxylin and eosin stains, or to improve evidence, from immunostaining mainly using the markers CD20, CD3, CD8, PNAd, or DC-LAMP (37). In addition, gene expression signatures that link the transcriptome to TLS presence have been generated (34, 44). Nonetheless, a large number of questions remains to be resolved regarding the composition and tumor-immunity functions of TLS. Thus far, B and T cells have been the main focus in the context of tumor-associated TLS. However, despite some initial insight, it remains unclear which B-cell states interact with which T-cell states. What is the frequency and precise role of Tregs, Tfh, and other CD4+ T-cell subsets observed in TLSs? How is tumor cell killing by CD8 T cells modified by TLSs? Many of the additional cell types are not well explored in the context of TLSs. *LAMP3* (DC-LAMP) expressing dendritic cells (DC) located in the T-cell–rich area of TLS promote T-cell responses (45). As these cells also derive from conventional DCs (46), it is unclear whether also FDCs have a prominent role as in lymph nodes. FDCs located within B-cell follicles have been reported using CD21 and CD23 staining, however, these receptors are not optimal markers as they can also be expressed by B cells (2, 47). FRCs may be surrogated by resident fibroblasts in tumor-associated TLSs. How the resulting fibroblast nets differs from those in the lymph node and how this modifies the interaction between immune cells, remains to be determined. Tingible-body macrophages, a GC-specific cell type, may also be present in a subset of TLSs. Also, HEVs have been reported (48, 49); however, it is unclear how lymphocyte recruitment is coordinated. In particular, a spatial map of cell types at high resolution would be illuminating. Such attempts to explore spatial transcriptomics are based on sequencing or imaging platforms (50). The methods are currently refined and promise to reveal TLS architecture in unprecedented detail.

Clinical Relevance of B Cells and TLS

The role of B cells and TLS for patient prognosis has been under considerable investigation in a variety of cancer types, with their presence predominantly conferring good outcomes to patients (4, 30, 51–57), for a comprehensive review see Kinker and colleagues (1). In addition, the well-known positive prognostic effect of T cells is stronger when B cells are present (58). So far, the impact of B cells and TLS in ICB is less well studied, yet several recent reports support a predictive role (**Table 1**).

Notably, three seminal studies recently published by Nature comprehensively described the importance of TLS in the context of ICB (2, 4, 30). Helmink and colleagues found B-cell–specific genes to be among the most discriminatory genes between responders and nonresponders in a neoadjuvant ICB-treated melanoma cohort [PD-1 or PD-1+CTLA4 inhibitors (CTLA4i)]. Moreover, a predefined B-cell signature (59) and TLS density were increased in responders both at baseline and early on-treatment samples. In an additional presurgical ICB trial of renal cell carcinoma, B-cell signature and TLS density were elevated in responders. The melanoma trial also revealed that responders contained more abundant and diverse BCR sequences, and in a subset of patients suggested a higher proportion of memory B cells in tumors of responders than nonresponders (2). Next, Petitprez and colleagues comprehensively reanalyzed gene expression data from sarcoma specimens to find five groups, thereof group "E" tumors displayed the highest immune cell signature expression and were associated with a favorable prognosis. In an independent cohort, group "E" tumors were linked to the presence of TLS. In a further clinical trial cohort using sarcoma tumors at baseline of PD-1 inhibitor (PD-1i) treatment, group "E" patients were enriched in responders to ICB (30). Finally, our group observed that the $CD20^+$ B-cell subset conferred a favorable prognosis to patients with metastatic melanoma. CD20+ B cells were in all cases surrounded by T cells and formed TLSs of varying maturity levels. Interestingly, in scRNAseq data, B-cell–rich tumors had an increase of naïve *TCF7*-expressing CD4 and CD8 T cells, indicating an influx of naïve T cells to the TLS. We established a TLS signature consisting of 9 genes, which was highly correlated to the expression of TLS hallmark and B-cell genes. In four ICB-treated melanoma cohorts, consisting of two CTLA4i-treated cohorts and two cohorts based on PD-1i treatment, a high score of the TLS signature in pretreatment samples was associated with favorable patient outcome (4).

Additional evidence of the impact of B-cell and TLS presence in the context of immunotherapy is presently emerging (**Table 1**). Thus, a signature of plasmablast-like B cells correlated with good outcomes to ICB in patients with melanoma (60). In pretreatment samples of a lung adenocarcinoma cohort, a B-cell signature correlating with the presence of TLS, was associated with a favorable outcome to ICB treatment (61). In a neoadjuvant PD-1i trial of resectable non–small cell lung cancer (NSCLC) cases, TLS and plasma cells were prevalent in the regression beds of responders (62). Moreover, in a bladder cancer trial of neoadjuvant PD-L1i/CTLA4i combination therapy, a higher TLS density in pretreatment tumor tissue correlated with favorable outcomes (63). In a further bladder cancer trial of preoperative PD-1i/CTLA4i combination therapy, TLS area did not correlate with response at baseline, however TLS induction was increased in on-treatment samples of responders (42). A further study on advanced-stage bladder cancer found that *CXCL13* expression is correlated with the presence of TLS and predicts favorable outcomes of PDL1i therapy (64). Finally, in a study that screened plasma from patients of various cancer types before ICB treatment, the protein most predictive of poor outcome, leukemia inhibitory factor (LIF), was negatively correlated to TLS presence (65). Interestingly, TLS density and size correlated with increased immune reactivation after PD-1 blockade in *ex vivo* patient derived tumor fragments, which in turn predicted patient response to PD-1i treatment (66). Recently, Vanhersecke and colleagues investigated pretreatment samples using PD-1i or PD-L1i therapy ($n = 328$) across a range of cancer types, with a larger portion from patients with lung cancer. Overall, 32% of samples contained TLSs, and TLSs were present in all cancer types (67). The presence of TLS was associated with good outcomes independent of CD8 T-cell density and PD-L1 expression. TLS presence also correlated with a good outcome in two validation cohorts (67). Notably, as ICB treatment is currently given at late localized or advanced disease stage, the majority of the described studies detected TLS from

Table 1. Initial studies discerning the effect of B cells and TLS on ICB treatment outcome.

Author (Year)	Cancer type	ICB treatment	Number of patients	Main finding	Impact outcome	Ref
Helmink *et al.* (2020)	Melanoma, Renal	Neoadjuvant/presurgical PD-1i or PD-1i+CTLA4i	21 (melanoma), 28 (renal)	B-cell signature and TLS density are elevated in responders	Positive	2
Cabrita *et al.* (2020)	Melanoma	PD-1i and/or CTLA4i	186 (4 cohorts)	High TLS signature score is associated with favorable outcomes	Positive	4
Petitprez *et al.* (2020)	Sarcoma	PD-1i	47	TLS-linked molecular group "E" is enriched in responders	Positive	30
Cottrell *et al.* (2018)	NSCLC	Neoadjuvant PD-1i	20	TLS and plasma cells are enriched in regression beds of responders	Positive	62
Budczies *et al.* (2021)	Lung adenocarcinoma	PD-1i or PD-L1i	43	TLS-linked B-cell signature is associated with favorable outcomes	Positive	61
Griss *et al.* (2019)	Melanoma	PD-1i	51	Plasmablast signature is associated with favorable outcomes	Positive	60
de Jonge *et al.* (2021)	Melanoma	CTLA4i	22	Circulating IL6/TNFα B cells are associated with poor outcomes	Negative	17
Gao *et al.* (2020)	Urothelial cancer	Neoadjuvant PD-L1i+CTLA4i	26	TLS density pretreatment is associated with favorable outcomes	Positive	63
van Dijk *et al.* (2020)	Urothelial cancer	Preoperative PD-1i+CTLA4i	24	TLS enrichment on-treatment is associated with response	Positive	42
Groeneveld *et al.* (2021)	Urothelial cancer	PD-L1i	348	TLS-linked CXCL13 expression correlates with favorable outcomes	Positive	64
Loriot *et al.* (2021)	Pan-cancer	PD-1i or PD-L1i based	59	TLS anti-correlated plasma LIF is associated with poor outcomes	Positive	65
Voabil *et al.* (2021)	Pan-cancer	PD-1i	33	TLS correlate to *ex vivo* immune reaction and patient response	Positive	66
Vanhersecke *et al.* (2021)	Pan-cancer (39% NSCLC)	PD-1i or PD-L1i	328 (discovery cohort)	Presence of mature TLS is associated with favorable outcomes	Positive	67

metastatic sites. Together, evidence from several cancer types is now accumulating that TLS in pretreatment samples confers favorable patient outcome from ICB.

So far, it is unclear how TLSs govern antitumor immunity under ICB treatment. Indeed, PD-L1 is not only expressed by tumor cells, but also by macrophages (68), dendritic cells (69), and other cells. Similarly, PD-1 expression is not exclusive to cytotoxic T cells, but has been found on macrophages (70), and relevantly, on Tfh cells (71) and B cells (72), suggesting that ICB may directly activate TLS. Notably, elevated immune cell expression (73), including B cells (2), was detected in on-treatment melanoma samples of ICB responders. However, further mechanistic insight on the role of these immune cell compartments is needed. Moreover, it is interesting that ICB was suggested to change the composition of peripheral blood B cells (74). Also, ICB plus radiotherapy increased GC formation in the tumor-draining lymph node in a murine model of HPV-related cancer (75). Furthermore, PD-1i treatment induced the expansion of CD8 T cells that was not observed before treatment, indicating that ICB response is driven by incoming T cells (76). While more evidence is needed, TLS may be instrumental to restart antitumor defense during ICB treatment by mounting a fresh adaptive immune response using incoming B and T cells.

Therapies Targeted at B-Cell Activation and TLS Induction

Given the premise that ICB works better in the context of B-cell– and TLS-rich tumors, the development of therapies that activate B cells and support the induction of TLS are highly desirable and can potentially improve the clinical outcome after ICB. However, our current limited understanding of TLS induction, formation, and maintenance have severely hampered the development of effective strategies. So far, strong and reproducible evidence on strategies that can strengthen B cell and TLSs in human cancers are lacking. Nonetheless, early investigations with promising findings have been described.

In preclinical models the administration of LIGHT-VTP, a compound with a dual ability to modulate tumor blood vessels and induce the formation of TLS, improved the sensitivity to ICB of immunotherapy-resistant rodent tumors (77). The combined treatment induced intratumoral activation of cytotoxic T cells leading to improved survival. The antitumor effects could be further improved when combined with anticancer vaccination. In apparent contrast with these findings, in another study, agonistic CD40 therapy induced TLS in preclinical models of glioma; however as a trade-off, this therapy markedly induced suppressive CD11b+ B cells which in turn impaired responses to ICB (38). Overall, these studies demonstrate that TLS formation can be induced even in the brain, and warrant further investigation into combinatorial strategies with TLS-inducing agents plus established immunotherapeutics such as ICB.

Another attractive strategy is to activate B cells with conventional treatments. In a recent study, following tumor-directed radiation, B cells in the tumor-draining lymph node had increased expression of MHC-II which may improve their APC function (75). In two independent studies, cancer vaccines have shown the ability to induce TLS: in HPV 16–positive cervical cancer, therapeutic vaccination targeting

HPV16 E6/E7 antigens induced organized TLS structures in the stroma subjacent to residual intraepithelial lesions, despite modest detectable responses in circulating T lymphocytes (78). In another study conducted in patients with pancreatic adenocarcinoma, an allogeneic granulocyte-macrophage colony-stimulating factor secreting vaccine given in combination with low dose cyclophosphamide induced TLS with a distinct Th17 signature (79). Furthermore, neoadjuvant chemotherapy has been found to affect B-cell populations in a breast cancer study (80). These studies demonstrated that B-cell activation and TLS formation can be achieved with treatments that are not directly designed to achieve this outcome.

Currently, the potential of generating inducible TLS with sophisticated technologies including novel biomaterials is a matter of continuous investigation, with highly promising preliminary results achieved in preclinical models [reviewed in (81)].

In summary, B-cell activation and TLS formation can be induced therapeutically. However, these findings appear still far from the reproducibility and robustness required for entering the mainstream of cancer treatment.

Perspective

TLS have been recognized as a key component of antitumor immunity. A deeper understanding of TLS development and function in tumors at the single cell and spatial level is expected to yield insights that will ultimately improve efforts to induce and modulate TLS. This way, tumors that maintain an immune poor or exhausted microenvironment may be exposed to a fresh local immune reaction. An advantage of TLS mediated immune response is the recruitment of incoming lymphocytes to the site, potentially invigorating tumor recognition and eradication. The data so far allow to hypothesize that TLS generation will improve immune checkpoint blockade therapies. Further, it is also conceivable that combinations with other therapies such as DC vaccines, T-cell transfer or conventional treatments like chemo- and radiotherapy, may overcome tumor immune evasion, and hence change clinical utility of ICB.

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References

1. Kinker GS, Vitiello GAF, Ferreira WAS, Chaves AS, Cordeiro de Lima VC, Medina TDS. B cell orchestration of antitumor immune responses: a matter of cell localization and communication. Front Cell Dev Biol 2021;9:678127.
2. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature 2020; 577:549–55.
3. Jerby-Arnon L, Shah P, Cuoco MS, Rodman C, Su MJ, Melms JC, et al. A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. Cell 2018;175:984–97e24.
4. Cabrita R, Lauss M, Sanna A, Donia M, Larsen MS, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature 2020;577:561–5.
5. Hu Q, Hong Y, Qi P, Lu G, Mai X, Xu S, et al. Atlas of breast cancer infiltrated B-lymphocytes revealed by paired single-cell RNA sequencing and antigen receptor profiling. Nat Commun 2021;12:2186.
6. Hollern DP, Xu N, Thennavan A, Glodowski C, Garcia-Recio S, Mott KR, et al. B cells and T follicular helper cells mediate response to checkpoint inhibitors in high mutation burden mouse models of breast cancer. Cell 2019; 179:1191–206.
7. Cui C, Wang J, Chen P-M, Connolly KA, Damo M, Fagerberg E, et al. Neoantigen driven B cell and CD4(+) T-follicular helper cell collaboration promotes robust antitumor CD8(+) T-cell responses. bioRxiv 2020:2020.12.23.424168.
8. Wieland A, Patel MR, Cardenas MA, Eberhardt CS, Hudson WH, Obeng RC, et al. Defining HPV-specific B-cell responses in patients with head and neck cancer. Nature 2021;597:274–8.
9. Biswas S, Mandal G, Payne KK, Anadon CM, Gatenbee CD, Chaurio RA, et al. IgA transcytosis and antigen recognition govern ovarian cancer immunity. Nature 2021;591:464–70.
10. Hu X, Zhang J, Wang J, Fu J, Li T, Zheng X, et al. Landscape of B-cell immunity and related immune evasion in human cancers. Nat Genet 2019;51:560–7.
11. Sharonov GV, Serebrovskaya EO, Yuzhakova DV, Britanova OV, Chudakov DM. B cells, plasma cells, and antibody repertoires in the tumor microenvironment. Nat Rev Immunol 2020;20:294–307.
12. Reuschenbach M, von Knebel Doeberitz M, Wentzensen N. A systematic review of humoral immune responses against tumor antigens. Cancer Immunol Immunother 2009;58:1535–44.
13. Shang J, Zha H, Sun Y. Phenotypes, functions, and clinical relevance of regulatory B cells in cancer. Front Immunol 2020;11:582657.
14. Michaud D, Steward CR, Mirlekar B, Pylayeva-Gupta Y. Regulatory B cells in cancer. Immunol Rev 2021;299:74–92.
15. Murakami Y, Saito H, Shimizu S, Kono Y, Shishido Y, Miyatani K, et al. Increased regulatory B cells are involved in immune evasion in patients with gastric cancer. Sci Rep 2019;9:13083.
16. Zhou X, Su YX, Lao XM, Liang YJ, Liao GQ. CD19(+)IL-10(+) regulatory B cells affect survival of tongue squamous cell carcinoma patients and induce resting CD4(+) T cells to CD4(+)Foxp3(+) regulatory T cells. Oral Oncol 2016;53:27–35.
17. de Jonge K, Tille L, Lourenco J, Maby-El Hajjami H, Nassiri S, Racle J, et al. Inflammatory B cells correlate with failure to checkpoint blockade in melanoma patients. Oncoimmunology 2021;10:1873585.
18. Dieu-Nosjean MC, Goc J, Giraldo NA, Sautes-Fridman C, Fridman WH. Tertiary lymphoid structures in cancer and beyond. Trends Immunol 2014; 35:571–80.
19. Rodriguez AB, Peske JD, Woods AN, Leick KM, Mauldin IS, Meneveau MO, et al. Immune mechanisms orchestrate tertiary lymphoid structures in tumors via cancer-associated fibroblasts. Cell Rep 2021;36:109422.
20. Colbeck EJ, Ager A, Gallimore A, Jones GW. Tertiary lymphoid structures in cancer: drivers of antitumor immunity, immunosuppression, or bystander sentinels in disease? Front Immunol 2017;8:1830.
21. Gu-Trantien C, Migliori E, Buisseret L, de Wind A, Brohee S, Garaud S, et al. CXCL13-producing TFH cells link immune suppression and adaptive memory in human breast cancer. JCI Insight 2017;2:e91487.
22. van der Leun AM, Thommen DS, Schumacher TN. CD8(+) T-cell states in human cancer: insights from single-cell analysis. Nat Rev Cancer 2020;20: 218–32.
23. Thommen DS, Koelzer VH, Herzig P, Roller A, Trefny M, Dimeloe S, et al. A transcriptionally and functionally distinct PD-1(+) CD8(+) T-cell pool with predictive potential in non–small cell lung cancer treated with PD-1 blockade. Nat Med 2018;24:994–1004.
24. Workel HH, Lubbers JM, Arnold R, Prins TM, van der Vlies P, de Lange K, et al. A transcriptionally distinct CXCL13(+)CD103(+)CD8(+) T-cell population is associated with B-cell recruitment and neoantigen load in human cancer. Cancer Immunol Res 2019;7:784–96.

25. Gago da Graca C, van Baarsen LGM, Mebius RE. Tertiary lymphoid structures: diversity in their development, composition, and role. J Immunol 2021;206:273–81.
26. de Chaisemartin L, Goc J, Damotte D, Validire P, Magdeleinat P, Alifano M, et al. Characterization of chemokines and adhesion molecules associated with T-cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res 2011;71:6391–9.
27. Weinstein AM, Storkus WJ. Biosynthesis and functional significance of peripheral node addressin in cancer-associated TLO. Front Immunol 2016;7:301.
28. De Silva NS, Klein U. Dynamics of B cells in germinal centers. Nat Rev Immunol 2015;15:137–48.
29. Kroeger DR, Milne K, Nelson BH. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. Clin Cancer Res 2016;22:3005–15.
30. Petitprez F, de Reynies A, Keung EZ, Chen TW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. Nature 2020;577:556–60.
31. Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am J Respir Crit Care Med 2014;189:832–44.
32. Calderaro J, Petitprez F, Becht E, Laurent A, Hirsch TZ, Rousseau B, et al. Intratumoral tertiary lymphoid structures are associated with a low risk of early recurrence of hepatocellular carcinoma. J Hepatol 2019;70:58–65.
33. Figenschau SL, Fismen S, Fenton KA, Fenton C, Mortensen ES. Tertiary lymphoid structures are associated with higher tumor grade in primary operable breast cancer patients. BMC Cancer 2015;15:101.
34. Messina JL, Fenstermacher DA, Eschrich S, Qu X, Berglund AE, Lloyd MC, et al. 12-Chemokine gene signature identifies lymph node–like structures in melanoma: potential for patient selection for immunotherapy? Sci Rep 2012;2:765.
35. Garcia-Hernandez ML, Uribe-Uribe NO, Espinosa-Gonzalez R, Kast WM, Khader SA, Rangel-Moreno J. A unique cellular and molecular microenvironment is present in tertiary lymphoid organs of patients with spontaneous prostate cancer regression. Front Immunol 2017;8:563.
36. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-term survival for patients with non–small cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 2008;26:4410–7.
37. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer 2019;19:307–25.
38. van Hooren L, Vaccaro A, Ramachandran M, Vazaios K, Libard S, van de Walle T, et al. Agonistic CD40 therapy induces tertiary lymphoid structures but impairs responses to checkpoint blockade in glioma. Nat Commun 2021;12:4127.
39. Lee M, Heo SH, Song IH, Rajayi H, Park HS, Park IA, et al. Presence of tertiary lymphoid structures determines the level of tumor-infiltrating lymphocytes in primary breast cancer and metastasis. Mod Pathol 2019;32:70–80.
40. Cipponi A, Mercier M, Seremet T, Baurain JF, Theate I, van den Oord J, et al. Neogenesis of lymphoid structures and antibody responses occur in human melanoma metastases. Cancer Res 2012;72:3997–4007.
41. Posch F, Silina K, Leibl S, Mundlein A, Moch H, Siebenhuner A, et al. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. Oncoimmunology 2018;7:e1378844.
42. van Dijk N, Gil-Jimenez A, Silina K, Hendricksen K, Smit LA, de Feijter JM, et al. Preoperative ipilimumab plus nivolumab in locoregionally advanced urothelial cancer: the NABUCCO trial. Nat Med 2020;26:1839–44.
43. Jansen CS, Prokhnevska N, Master VA, Sanda MG, Carlisle JW, Bilen MA, et al. An intratumoral niche maintains and differentiates stem-like CD8 T cells. Nature 2019;576:465–70.
44. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, et al. CD4(+) follicular helper T-cell infiltration predicts breast cancer survival. J Clin Invest 2013;123:2873–92.
45. Goc J, Germain C, Vo-Bourgais TK, Lupo A, Klein C, Knockaert S, et al. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res 2014;74:705–15.
46. Cheng S, Li Z, Gao R, Xing B, Gao Y, Yang Y, et al. A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. Cell 2021;184:792–809.
47. Wennhold K, Thelen M, Lehmann J, Schran S, Preugszat E, Garcia-Marquez M, et al. CD86(+) Antigen-presenting B cells are increased in cancer, localize in tertiary lymphoid structures, and induce specific T-cell responses. Cancer Immunol Res 2021;9:1098–108.
48. Avram G, Sanchez-Sendra B, Martin JM, Terradez L, Ramos D, Monteagudo C. The density and type of MECA-79–positive high endothelial venules correlate with lymphocytic infiltration and tumor regression in primary cutaneous melanoma. Histopathology 2013;63:852–61.
49. Martinet L, Le Guellec S, Filleron T, Lamant L, Meyer N, Rochaix P, et al. High endothelial venules (HEV) in human melanoma lesions: major gateways for tumor-infiltrating lymphocytes. Oncoimmunology 2012;1:829–39.
50. Rao A, Barkley D, Franca GS, Yanai I. Exploring tissue architecture using spatial transcriptomics. Nature 2021;596:211–20.
51. Bruni D, Angell HK, Galon J. The immune contexture and immunoscore in cancer prognosis and therapeutic efficacy. Nat Rev Cancer 2020;20:662–80.
52. Rodriguez AB, Engelhard VH. Insights into tumor-associated tertiary lymphoid structures: novel targets for antitumor immunity and cancer immunotherapy. Cancer Immunol Res 2020;8:1338–45.
53. Di Caro G, Bergomas F, Grizzi F, Doni A, Bianchi P, Malesci A, et al. Occurrence of tertiary lymphoid tissue is associated with T-cell infiltration and predicts better prognosis in early-stage colorectal cancers. Clin Cancer Res 2014;20:2147–58.
54. Hennequin A, Derangere V, Boidot R, Apetoh L, Vincent J, Orry D, et al. Tumor infiltration by Tbet+ effector T cells and CD20+ B cells is associated with survival in gastric cancer patients. Oncoimmunology 2016;5:e1054598.
55. Hiraoka N, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favorable prognosticator in patients with pancreatic cancer. Br J Cancer 2015;112:1782–90.
56. Rakaee M, Kilvaer TK, Jamaly S, Berg T, Paulsen EE, Berglund M, et al. Tertiary lymphoid structure score: a promising approach to refine the TNM staging in resected non-small cell lung cancer. Br J Cancer 2021;124:1680–9.
57. Wirsing AM, Ervik IK, Seppola M, Uhlin-Hansen L, Steigen SE, Hadler-Olsen E. Presence of high-endothelial venules correlates with a favorable immune microenvironment in oral squamous cell carcinoma. Mod Pathol 2018;31:910–22.
58. Wouters MCA, Nelson BH. Prognostic significance of tumor-infiltrating B cells and plasma cells in human cancer. Clin Cancer Res 2018;24:6125–35.
59. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol 2016;17:218.
60. Griss J, Bauer W, Wagner C, Simon M, Chen M, Grabmeier-Pfistershammer K, et al. B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. Nat Commun 2019;10:4186.
61. Budczies J, Kirchner M, Kluck K, Kazdal D, Glade J, Allgauer M, et al. A gene expression signature associated with B cells predicts benefit from immune checkpoint blockade in lung adenocarcinoma. Oncoimmunology 2021;10:1860586.
62. Cottrell TR, Thompson ED, Forde PM, Stein JE, Duffield AS, Anagnostou V, et al. Pathologic features of response to neoadjuvant anti–PD-1 in resected non–small cell lung carcinoma: a proposal for quantitative immune-related pathologic response criteria (irPRC). Ann Oncol 2018;29:1853–60.
63. Gao J, Navai N, Alhalabi O, Siefker-Radtke A, Campbell MT, Tidwell RS, et al. Neoadjuvant PD-L1 plus CTLA-4 blockade in patients with cisplatin-ineligible operable high-risk urothelial carcinoma. Nat Med 2020;26:1845–51.
64. Groeneveld CS, Fontugne J, Cabel L, Bernard-Pierrot I, Radvanyi F, Allory Y, et al. Tertiary lymphoid structures marker CXCL13 is associated with better survival for patients with advanced-stage bladder cancer treated with immunotherapy. Eur J Cancer 2021;148:181–9.
65. Loriot Y, Marabelle A, Guegan JP, Danlos FX, Besse B, Chaput N, et al. Plasma proteomics identifies leukemia inhibitory factor (LIF) as a novel predictive biomarker of immune checkpoint blockade resistance. Ann Oncol 2021;32:1381–90.
66. Voabil P, de Bruijn M, Roelofsen LM, Hendriks SH, Brokamp S, van den Braber M, et al. An *ex vivo* tumor fragment platform to dissect response to PD-1 blockade in cancer. Nat Med 2021;27:1250–61.
67. Vanhersecke L, Brunet M, Guégan J-P, Rey C, Bougouin A, Cousin S, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. Nature Cancer 2021;2:794–802.
68. Liu Y, Zugazagoitia J, Ahmed FS, Henick BS, Gettinger SN, Herbst RS, et al. Immune cell PD-L1 colocalizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy. Clin Cancer Res 2020;26:970–7.
69. Peng Q, Qiu X, Zhang Z, Zhang S, Zhang Y, Liang Y, et al. PD-L1 on dendritic cells attenuates T-cell activation and regulates response to immune checkpoint blockade. Nat Commun 2020;11:4835.

70. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumor-associated macrophages inhibits phagocytosis and tumor immunity. Nature 2017;545:495–9.
71. Shi J, Hou S, Fang Q, Liu X, Liu X, Qi H. PD-1 controls follicular T helper cell positioning and function. Immunity 2018;49:264–74.
72. Thibult ML, Mamessier E, Gertner-Dardenne J, Pastor S, Just-Landi S, Xerri L, et al. PD-1 is a novel regulator of human B-cell activation. Int Immunol 2013;25: 129–37.
73. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell 2017;171:934–49.
74. Das R, Bar N, Ferreira M, Newman AM, Zhang L, Bailur JK, et al. Early B cell changes predict autoimmunity following combination immune checkpoint blockade. J Clin Invest 2018;128:715–20.
75. Kim SS, Shen S, Miyauchi S, Sanders PD, Franiak-Pietryga I, Mell L, et al. B cells improve overall survival in HPV-associated squamous cell carcinomas and are activated by radiation and PD-1 blockade. Clin Cancer Res 2020;26: 3345–59.
76. Yost KE, Satpathy AT, Wells DK, Qi Y, Wang C, Kageyama R, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. Nat Med 2019; 25:1251–9.
77. Johansson-Percival A, He B, Li ZJ, Kjellen A, Russell K, Li J, et al. De novo induction of intratumoral lymphoid structures and vessel normalization enhances immunotherapy in resistant tumors. Nat Immunol 2017;18:1207–17.
78. Maldonado L, Teague JE, Morrow MP, Jotova I, Wu TC, Wang C, et al. Intramuscular therapeutic vaccination targeting HPV16 induces T-cell responses that localize in mucosal lesions. Sci Transl Med 2014;6:221ra13.
79. Lutz ER, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res 2014;2:616–31.
80. Lu Y, Zhao Q, Liao JY, Song E, Xia Q, Pan J, et al. Complement signals determine opposite effects of B cells in chemotherapy-induced immunity. Cell 2020;180: 1081–97.
81. Aoyama S, Nakagawa R, Mule JJ, Mailloux AW. Inducible tertiary lymphoid structures: promise and challenges for translating a new class of immunotherapy. Front Immunol 2021;12:675538.