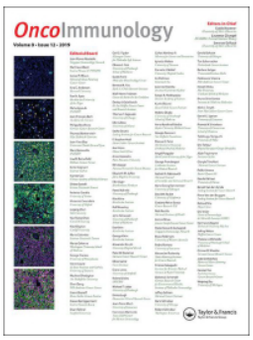

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REVIEW

Tertiary lymphoid structures and B lymphocytes in cancer prognosis and response to immunotherapies

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ABSTRACT

Tertiary lymphoid structures (TLS) are ectopic cellular aggregates that resemble secondary lymphoid organs in their composition and structural organization. In contrast to secondary lymphoid organs, TLS are not imprinted during embryogenesis but are formed in non-lymphoid tissues in response to local inflammation. TLS structures exhibiting a variable degree of maturation are found in solid tumors. They are composed of various immune cell types including dendritic cells and antigen-specific B and T lymphocytes, that together, actively drive the immune response against tumor development and progression. This review highlights the successive steps leading to tumor TLS formation and its association with clinical outcomes. We discuss the role played by tumor-infiltrating B lymphocytes and plasma cells, their prognostic value in solid tumors and immunotherapeutic responses and their potential for future targeting.

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Introduction

The composition and quality of tumor infiltrating immune cells directly dictates patient's outcomes and therapeutic efficiencies.[1] Strong anti-tumor immune responses are achieved through the interplay between innate and adaptive immune cells which drive the expansion and activation of tumor antigen-specific cytotoxic T cells and the production of antibodies by plasmablasts and plasma cells (collectively termed antibody-secreting cells (ASCs)).[2] However, the latter has not been extensively investigated in the tumor context until recently.[3–5] At steady-state, secondary lymphoid organs (SLO) act as central hubs where dynamic immune cell interactions can occur continuously between sentinel cells, such as dendritic cells, which migrate to the lymph nodes through dedicated vessels, called high endothelial venules (HEV),[6] and lymph node-resident immune lymphocytes (B and T cells). These innate–adaptive immune cell interactions allow screening of the body surfaces for detection of, and appropriate response to, potential immune threats. The coordination of optimal cell positioning to enable these interactions depends on chemokines that control the migratory patterns of these cells.[7] When a threat is detected, such as an epithelial cell transformation that may lead to tumor development, the local immune response may be insufficient, and the generation of an efficient adaptive immune response then relies on the capacity of activated dendritic cells to migrate to the closest draining lymph node and to present MHC-antigen-derived peptide complexes to $CD4^+$ and $CD8^+$ T cells leading to antigen-specific T cell expansion and activation.[8] In addition, the production of antibodies results from the activation of antigen-specific B cells following cognate interactions with $CD4^+$ T cells to help drive B cell expansion, germinal center formation and ASC differentiation.[9–11]

In cancer, these effector cells then egress the regional lymph node to infiltrate the tumor microenvironment where they can pinpoint and eradicate cancer cells. Several groups have revealed that, in addition to this pathway, in some tumors, an adaptive immune response is generated *in situ* that mirrors the pattern normally associated with SLO. This happens within spatially well-organized structures called tertiary lymphoid structures (TLS). *De novo* TLS formation in tumors requires optimal cytokine and chemokine concentration and specialized immune cell types.[12] TLS formation can occur at both the margins and in the core of tumors. Similar to SLOs, mature TLS are composed of T and B cell zones and germinal centers. These compartmentalized structures contain innate immune cells and adaptive lymphocytes and may include dendritic cells, neutrophils, macrophages, helper $CD4^+$ and cytotoxic $CD8^+$ T lymphocytes, B cells, plasmablasts and plasma cells.[11,13] In addition, HEV often colocalizes with TLS in tumors allowing the initial immune cell recruitment, but also the egress of activated immune cells from the TLS to the circulation.[14,15]

Our current understanding of the local anti-tumor immune response is still fairly limited. For example, in melanoma, it is not unusual to observe partial or even complete spontaneous regression of primary tumors indicative of a potent ongoing endogenous anti-tumor immune response. However, regression of metastatic melanoma lesions is extremely rare, suggesting that the emergence of antigen-loss tumor variants that have invaded distant organs overwhelms the initial anti-tumor immune response.[16] Thus, we can ask how is such a potent outcome achieved early during tumor formation? What are the local cellular and molecular events that lead to primary tumor eradication? Does a tumor disappearance correlate with speci-

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fic structures, such as TLS, that are engendered by tumor development? Furthermore, what are the exact mechanisms involved? and, most importantly, can we increase or induce effective anti-tumor immune responses early in a response to drive stronger, or complete, tumor eradication before the spread of disease to distant organs? Successful cooperation between tumor-infiltrating innate and adaptive immune cells within TLS is certainly key to fruitful anti-tumor responses. Such outcomes appear likely to be achievable in the near future given our increased understanding of local and systemic immune responses through the use of cutting-edge technologies, sophisticated models and the development of innovative immunotherapies.

In this review, we explore the cellular and molecular requirements for TLS formation and highlight the role and impact of these ectopic lymphoid structures in cancer. As tumor B lymphocyte infiltration is closely linked to the presence of intra-tumoral TLS, we further detail the prognostic and therapeutic predictive value of B cells and ASCs in tumors, particularly in light with the latest findings in melanoma, renal cell carcinoma and sarcoma tumors.[3–5] We will discuss the possibility to further enhance anti-tumor immune responses by increasing TLS formation and targeting B cells and the antibody response in tumors.

1. Tertiary lymphoid structures – formation and composition

TLS formation – paralleling SLO development

TLS formation necessitates the cooperation of stromal cells with innate and adaptive immune cells, that together are positioned within the tissues to induce TLS neogenesis.[12] Similar to SLO formation, TLS generation might requires the local accumulation of CXCL13, RANKL and interleukin(IL)-7, which together recruit and activate lymphoid tissue-inducer cells (LTi)[17–20] (Figure 1). LTi cells interact with stromal cells through the pairing of lymphotoxin (LT) α1β2, expressed on the surface of LTi cells, with its receptor (LTβR) expressed on stromal cells. Interleukin (IL)-17 production by LTi cells[21] together with LTα1β2-LTR interaction induce *de novo* secretion of chemokines, angiogenic growth factors and the expression of adhesive molecules by IL-17R$^+$ stromal cells.[22] The expression of some of these factors is further amplified by additional interactions with other cell types, such as dendritic cells, CD8$^+$ T cells or natural killer (NK) cells, culminating in the secretion of VEGFA, VEGFC, essential for HEV formation, the production of CXCL12, CXCL13, CCL19 and CCL21, for immune cell recruitment and the expression of ICAM1, VCAM1 and MADCAM1 for cell retention[18,23–30] (Figure 1). Of note, mice deficient for Rorγt (which fail to develop LTi cells), Cxcl13 and IL-7 Rα (*Cxc13*$^{-/-}$ × *Il7ra*$^{-/-}$ mice) or lymphotoxin α (*Lta*$^{-/-}$ mice) expression, lack SLO formation, [20,31,32] although some lymphoid tissues such as the nasal-associated tissue (NALT)[33] or the tear duct-associated lymphoid tissue (TALT)[34] develop in the absence of Rorγt, LTβR or IL-7 R signaling. Collectively, these studies demonstrate that while SLO formation relies on critical cellular and molecular pathways during embryogenesis for their formation, post-natal development of lymphoid structures such as NALT or TALT does not follow these rules. This raises the question whether TLS development, which occurs after birth in chronic inflammatory responses, also depends on these pathways or alternatively, may use different cellular and molecular mechanisms for their initiation and formation.

Is SLO the right model?

In various inflammatory contexts, B cells,[35] macrophages[36] or IL-17 expressing T cells[37] can induce *de novo* TLS formation even when mice are deficient for LTi cells and have defective SLO. In mucosal tissues such as the gut, a fine balance between pro- and anti-inflammatory immune cells and signaling molecules is necessary to control microbiota diversity and composition and to maintain tissue homeostasis. *Roryt*-deficient mice

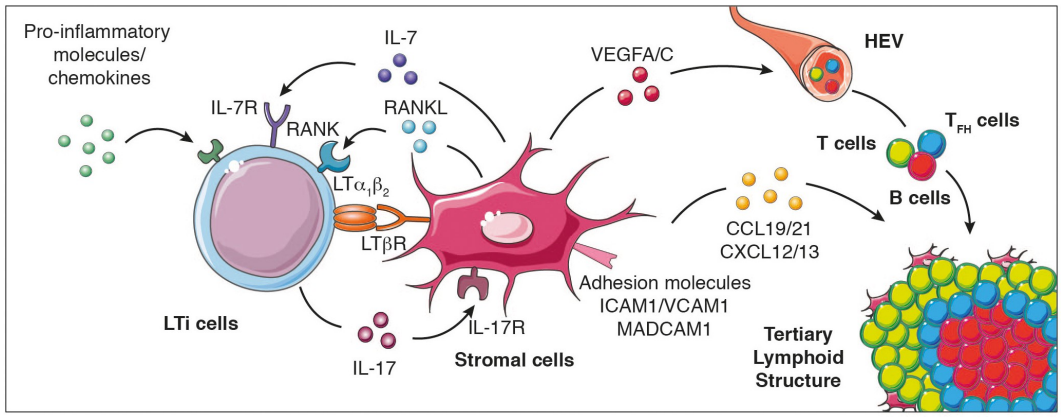

Figure 1. Cellular and molecular signals that control TLS formation. The local accumulation of pro-inflammatory molecules and chemokines promotes the recruitment of LTi cells to the inflammed site promoting their interaction with stromal cells to initiate TLS genesis and cytokine (IL-7, IL-17, RANKL, and LTα1β2) and cytokine receptor (IL-7R, IL-17R, RANK and LTβR) expression. When LTi cells are absent, other immune cells such as macrophages, B lymphocytes and Th17 cells can also interact with stromal cells to induce TLS formation. This interaction culminates in the production of chemokines (CCL19, CCL21, CXCL12 and CXCL13), pro-angiogenic molecules (vascular endothelial growth factors VEGFA and VEGFC) and the expression of adhesion molecules which facilitate the recruitment of additional immune cell types, their retention and organization into the nascent TLS. LTi cells, lymphoid tissue-inducer cells; Th17 cells, T helper cells secreting IL-17; LT, lymphotoxin; RANK, receptor activator of nuclear factor-κB; ICAM, intercellular adhesion molecule 1; VCAM1, Vascular adhesion molecule 1; MADCAM, mucosal vascular addressin cell adhesion molecule 1; VEGFC, vascular endothelial growth factor A/C; IL, interleukin; CCL19: C-C motif chemokine ligand 19; CXCL13, C-X-C motif chemokine ligand 13; HEV, high endothelial venules.

have impaired intestinal SLO development resulting in the absence of mesenteric lymph nodes and Peyer's and colonic patches, but also have defective cryptopatches and isolated lymphoid follicles.[32,38] While these lymphoid structures are critical to mount appropriate immune responses, LTi-deficient mice are still able to preserve their barrier integrity and to maintain intestinal homeostasis, as TLS development occurs in the colon of *Rorγt*-deficient mice during inflammation and seems to be dependent on the gut microbiota.[39] Similarly, inducible bronchus-associated lymphoid tissue (iBALT) develops in lungs after LPS exposure or influenza-infection in mice lacking SLO.[37,40] In influenza-infected *Lta*$^{-/-}$ mice, local CXCL13 and CCL21 expression colocalizes with the B cell-rich zone and PNAd-expressing HEVs, respectively, in iBALT.[40] Similarly, *Rorc*$^{-/-}$ and *Id2*$^{-/-}$ mice exposed to LPS and infected with influenza also form iBALT.[37] Together, these results demonstrate that local inflammation is a principal trigger of TLS formation, and similar mechanisms are likely to occur in tumors.[41] These vascular structures together with local chemotactic factors allow the recruitment and accumulation of B and T cells sustaining the initial formation and the assembly of the nascent TLS.[25,42] Overall, while a parallel between SLO and TLS formation occurs, additional molecular and cellular events principally dependent on sustained local inflammation trigger TLS neogenesis.

Temporal development and recruitment of cells in TLS

The temporal development and recruitment of cells to intratumoral TLS are, to date, largely unknown. Recently, Meylan and colleagues examined TLS formation in preneoplastic hepatic lesions from cirrhotic livers to determine whether TLS neogenesis was induced in early hepatic lesions and its associated immune profile.[43] This group found that a quarter of these preneoplastic hepatic lesions display TLS in cirrhotic nodules.[43] The presence of these structures was associated with increased densities of T cells, B cells and mature dendritic cells but lack CD21$^+$ follicular dendritic cells, indicating that most TLS consisted of immature lymphocytic aggregates rather than fully developed follicles.[43] They further identified the presence of immunosuppressive genes in lesions where TLS were found suggesting that inhibitory signals that emerge within cirrhotic nodules potentially inhibit ongoing immune responses.[43] However, for ethical and practical reasons, it was impossible to correlate patient clinical outcomes with the presence of these TLS in preneoplastic hepatic lesions. Nevertheless, this study has been critical in pinpointing that TLS formation is likely to occur very early during tumor development, in parallel with the early development of inflammation.

TLS maturation, composition and diversity

TLS composition encompasses both innate and adaptive immune cells that are surrounded by HEV. Using immunohistochemistry, early studies in melanoma[44] and non-small lung cancer[45] found tumor-associated structures resembling SLO that are composed of T cells (CD3$^+$), mature DCs (DC-LAMP$^+$) and follicular B cells (CD20$^+$) (Figure 2). The immune cell composition of these structures differs, ranging from disorganized cellular aggregates, often referred as immature or early TLS, to well-organized and structured organs containing follicles, mirroring SLO and populated by germinal centers and tumor antigen-specific T and B cells (Figure 2). Recent analyses used additional markers to better characterize the immune composition of TLS and follicles. Studies identified proliferative (Ki67$^+$) germinal center B cells (CD23$^+$) expressing AID, involved in somatic hypermutations and class switch recombination, as well as Bcl6, a transcription factor involved in germinal center B cell maturation (Figure 2).[46–48] In addition, follicular dendritic cells (CD21$^+$ or CD23$^+$) were detected within germinal centers with macrophages (CD68$^+$), CD4$^+$ and CD8$^+$ T cells (CD3$^+$), follicular CD4$^+$ T cells (Bcl6$^+$PD-1$^+$ICOS$^+$IL-21$^+$) and plasma cells (CD38$^+$CD138$^+$) often surrounding germinal centers indicative of an ongoing humoral and cytotoxic immune responses[46,49] (Figure 2).

2. Tertiary lymphoid structures in cancer – prognostic and predictive values

TLS have been detected in numerous tumor types using immunohistochemistry or chemokine gene signatures which tightly correlated with the presence of TLS identified by immunochemistry.[41,50–52] In particular, a 12-chemokine gene signature was correlated to increased survival in melanoma,[50] colorectal[51,52] and breast[53] cancers. In addition, other gene signatures containing 8 and 19 genes related to the presence of T follicular helper cells, type 1 helper CD4$^+$ T cells and B cells were reported in breast[54] and gastric[55] cancers. Collectively, this has led to the detection of TLS in lung,[41,45,56,57] oral squamous cell carcinoma,[58] breast,[54,59–61] colon,[51,62] stomach,[55,63,64] liver,[65] sarcoma,[3,66,67] bladder,[68] clear cell renal cell carcinoma,[4] ovarian cancer[46,69] and melanoma[4,5,15,44,50,70] (Table 1). TLS formation and densities vary between tumor types, and between patients. While the presence of TLS are largely associated with favorable outcomes, other studies do not see this positive association between TLS and patient prognosis (Table 1). These inconsistencies within a tumor type might be explained by TLS location (tumor core versus stroma or invasive margin), tumor stage (primary versus metastatic lesions), tumor subtype (e.g., highly or lowly mutated tumors), patient treatment history which may influence immune cell infiltration (e.g., immunogenic chemotherapy) or TLS immune cell composition diversity, particularly in T and B cell subsets[71] (Table 1). In addition, several studies have reported the positive association between the presence of TLS and B lymphocytes, and therapy responses. Particularly, three seminal studies demonstrated the beneficial impact of TLS and B lymphocytes in melanoma, renal cell carcinoma and sarcoma tumors with response to immune checkpoint blockers.[3–5] This observation has been recently extended to patients with advanced urothelial cancer who received combination of anti-PD-1 and anti-CTLA-4 antibodies before tumor resection.[72] In this setting, while no correlation was observed between the presence of TLS at baseline and therapy response, all patients who experienced pathological complete responses had enriched TLS after

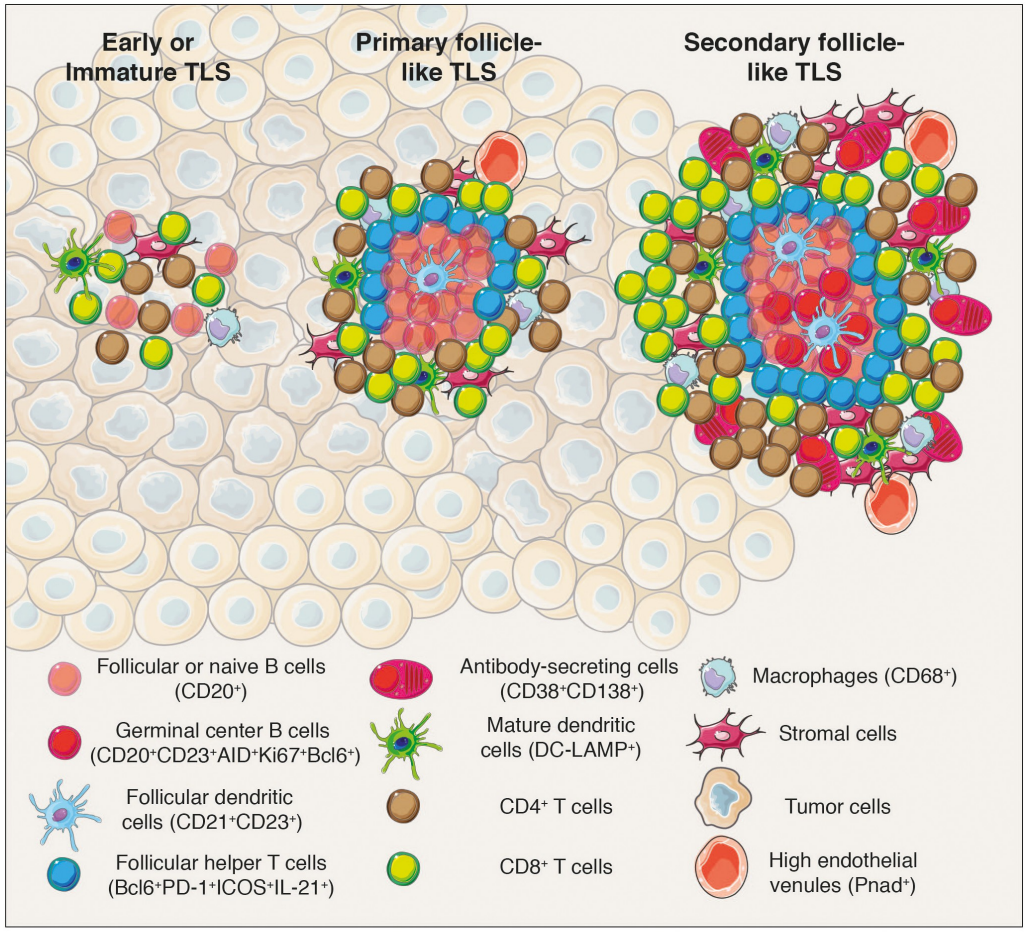

Figure 2

Figure 2. Different levels of TLS maturation and their composition. Tumor-associated TLS are heterogenous and range from poorly-organized cellular aggregates (Early or immature TLS) to well-organized structures forming primary follicles or secondary follicles containing germinal centers surrounded by specific vessels called high endothelial venules (PNad$^+$). Their cellular composition include stromal cells, innate and adaptive immune cells. In most of the analyses performed, cellular composition has been determined using immunofluorescence or immunochemistry analyses and relies on the expression of cell-specific markers to identify the cell types that form TLS. Mature secondary follicle-like TLS harbor a germinal center composed of proliferating mature germinal center B lymphocytes (CD20$^+$CD23$^+$AID$^+$Ki67$^+$Bcl6$^+$) and follicular dendritic cells (CD21$^+$CD23$^+$) surrounded by naïve or follicular B cells (CD20$^+$) and bordered by follicular helper T cells (Bcl6$^+$PD-1$^+$ICOS$^+$IL-21$^+$). In addition, TLS are formed of CD4$^+$ and CD8$^+$ T cells (CD3$^+$), plasma cells (CD38$^+$CD138$^+$), mature dendritic cells (DC-LAMP$^+$) and macrophages (CD68$^+$). CD, cluster of differenciation; DC-LAMP, dendritic cell lysosomal associated membrane glycoprotein; PD-1, programmed cell death 1; ICOS, Inducible costimulator; AID, activation-induced deaminase; Bcl6, B cell lymphoma 6 protein; PNad, peripheral node addressin.

treatment.[72] Thus, TLS could be induced during immune checkpoint treatment and favored the generation of local anti-tumor immune responses.

3. B lymphocytes take the center stage in anti-tumor immune responses

Unlike their T cell kin, the contribution of the B lymphocytes (B cells and their progeny, the ASC) to the antitumor immune response has only been acknowledged recently as their presence has been increasingly reported in a wide variety of tumors[11] (Table 2). Unexpectedly, both beneficial and detrimental roles were described, and their exact function thus remains unclear (Table 2). This complexity stems from the failure to appreciate the diversity of the B cell lineage, and the often limited characterization of the tumor-associated B lymphocytes. Accumulating evidence now points to substantial heterogeneity among B lymphocytes found within the tumor infiltrates, particularly in TLS, both in terms of maturation status and effector functions. B lymphocytes are the sole producers of antibodies making them critical to humoral immunity, but they also influence other immune and nonimmune cell subsets through the production of various cytokines and cellular mediators in the tumor microenvironment. For example, they secrete interferon (IFN)-γ and IL-12[93] which may promote cytotoxic CD8$^+$ T cell responses. In contrast, particularly in tumors, B lymphocytes may produce immunosuppressive molecules triggering regulatory T cell development and differentiation of myeloid-derived suppressor cells (MDSCs).[121,122] An emerging model posits that the divergent prognostic outcomes linked B cells could be reconciled by delineating subpopulations and clonality with pro- and anti-tumor roles.

B lymphocyte properties associated with anti-tumor responses

From the perspective of antitumor responses, the IgG1 antibody subclass exerts the most efficient effector activity. It fixes

Table 1. The prognostic value of TLS in cancers.

Cancer type	Tumor subtype	Tumor localization	Treatment history	TLS localization	TLS composition	Prognostic value	Predictive value	Refs
Non-small cell lung	Adenocarcinoma, squamous cell carcinoma and large cell carcinoma	Primary tumors	N.A.	Ti-BALT	$CD4^+$ and $CD8^+$ T cells, FDC ($CD21^+$), mature DC (DC-$Lamp^+$) and Plasma cells ($CD138^+$)	N.D.	N.A.	41
Non-small cell lung	Adenocarcinoma, squamous cell carcinoma	Primary tumors	Naive	Ti-BALT	B cells ($CD20^+$), mature DC (DC-$Lamp^+$), $CD3^+$ T cells and FDC ($CD21^+$)	Favorable (DC-Lamp high); No impact of other markers	N.A.	45
Non-small cell lung	Adenocarcinoma, squamous cell carcinoma and other subtypes	Primary tumors(2 cohorts)	Naive and neo-adjuvant chemotherapy	Ti-BALT	mature DC (DC-$Lamp^+$), $CD3^+$ T cells, macrophages (CD68+), B cells ($CD20^+$) and plasma cells ($CD138^+$) – germinal centers ($CD23^+IgD^-AID^+Bcl6^+$)	Favorable in both cohorts (DC-$Lamp^+$ and $CD20^+$)	N.A.	56
Non-small cell lung	Adenocarcinoma, squamous cell carcinoma and other subtypes	Primary tumors	Naive	Tumor	H&E staining – TLS and germinal centers – $CD3^+$ T cells and B cells ($CD20^+$)	Patients with higher numbers of TLS or B cells in their tumors have increased overall survival	N.A.	57
Oral squamous cell carcinoma	Tongue, bucca, gingiva and other locations	Primary tumors	Naive	Tumor	H&E staining – TLS and germinal centers – $CD3^+$ T cells, B cells ($CD20^+$), mature DC (DC-$Lamp^+$) and $PNAd^+$ HEV like vessels	Favorable	N.A.	58
Breast	ER^-HER2^-; $HER2^+$; ER^+HER2^+	Primary tumors	2 cohorts – one treated with neoadjuvant chemotherapy	Tumor	Tfh cell signature and *CXCL13* gene expression	Favorable	Associated with a higher rate of pathological complete response	54
Breast	$HER2^+$	Primary tumors	Chemotherpay and trastuzumab	Peri-tumoral tissue	H&E staining – TLS in adjacent tissue and germinal centers	N.D.	No impact of TLS. Increased disease free-survival with moderate to abundant tumor infiltrating lymphocytes	59
Breast	ER, PR and $HER2^{\pm}$	Primary tumors	Naive	Tumor and peri-tumoral tissue	$CD3^+$ $CD4^+$ and $CD8^+$ T cells, B cells ($CD20^+$), FDC ($CD21^+$) – germinal centers ($Bcl6^+$), $PNAd^+$ HEV like vessels	Associated with aggressive tumors	N.A.	60
Breast	TNBC	Primary tumors	Naive	Tumor and peri-tumoral tissue	H&E staining – $PNAd^+$ HEV like vessels	Favorable	N.A.	61
Colon	Left and right segments, rectum and sigmoid. MSI-H and MSS	Primary tumors	Naive and treated patients	Tumor and peri-tumoral tissue	$CD3^+$ T cells, B cells ($CD20^+$ and $CD79a^+$), FDC ($CD21^+$) and gene signature	Favorable	N.A.	51
Colon	Colon and rectum, MSI-H and MSS	Primary tumors	Naive	Invasive margin	$CD3^+$ T cells, B cells ($CD20^+$), FDC ($CD21^+$), CCL21 and CXCL13, $PNAd^+$ HEV like vessels and $Lyve\text{-}1^+$ lymphatic vessels	Favorable for Stage II patients (without lymph node metastases). No impact for Stage II patients	N.A.	62
Stomach	Intestinal type (Lauren class) and *H. Pylori* $^{\pm}$.	Primary tumors	N.A.	Tumor	$CD3^+$ T cells, B cells ($CD20^+$) and gene signature (*CXCL13, CCL19* and *CCL21*)	Associated with advanced disease	N.A.	63
Stomach	Intestinal, mixte and Signet ring cells	Primary tumors	Naive and neoadjuvant chemotherapy	Tumor and invasive margin	$CD3^+$ T cells, B cells ($CD20^+$), mature DC (DC-$Lamp^+$) and $PNAd^+$ HEV like vessels	Tbet $CD20^{high}$ associated with a favorable outcome	N.A.	55

(*Continued*)

Table 1. (Continued).

Cancer type	Tumor subtype	Tumor localization	Treatment history	TLS localization	TLS composition	Prognostic value	Predictive value	Refs
Stomach	Differentiated and undifferentiated	Primary tumors	Naive	Tumor and invasive margin	$CD3^+$ T cells, B cells ($CD20^+$), plasma cells ($CD138^+$), and FDC ($CD21^+$).	Favorable	N.A.	64
Liver	Hepatocellular carcinoma	Primary tumors	Naive	Tumor	Hematein-eosin-saffron stained slides and genes signature	Presence of intr-tumoral TLS associated with a lower risk of early relapse	N.A.	65
Sarcoma	Six different histology types (DDLPS, LMS, UPS, Synovial sarcoma, Myxoid liposarcoma and GIST)	Primary tumors	N.A.	Tumor	Immunofluorescence and genes signature	B cells and TLS are both associated with favorable outcomes	Patients in Class E (high density of B cells and presence of TLS) exhibited the highest response rate to PD-1 blockade treatment	3
Sarcoma	Three different histology types (UPS, ERMS and ARMS)	Primary tumors and metastases	Naive and pretreated patients	Tumor	$CD3^+$ $CD8^+$ and $Foxp3^+$ T cells, $CD163^+$ macrophages, $CD31^+$ endothelial cells and B cells ($CD20^+$)	No impact of T cell and macrophage densities or the presence of TLS with clinical outcomes	N.A.	66
Sarcoma	GIST	Primary tumors	Naive	Tumor	Multiplex immunohistochemistry	Presence of TLS associated with smaller tumor size, reduced relapse and increased survival	Presence of TLS are associated with reduced probability of future development of imatinib resistances	67
Kidney	Various histological subtypes	Primary tumors and metastases	Naive and pretreated patients	Tumor	Genes signature	No impact of B cell-lineage	B cell signature presence of TLS associated with response to immunotherapy	4
Bladder	pT1 bladder cancer	Primary tumors	Naive	Tumor and peri-tumoral tisue	$CD3^+$ and $CD8^+$ T cells	Associated with advanced disease and poor prognosis	N.A.	68
Ovarian	High-grade serous ovarian cancer	Primary tumors	Naive	Tumor stroma	$CD3^+$ and $CD8^+$ T cells, $CD20^+$ B cells, $CD21^+$ FDC and $CD38^+CD138^+CD79a^+$ plasma cells	High tumor-infiltration with plasma cells are associated with increased survival	N.A.	46
Ovarian	High-grade serous ovarian cancer	Primary tumors	Naive	Tumor stroma	B cells ($CD20^+$) and mature DC ($DC\text{-}Lamp^+$)	Favorable	N.A.	
Skin	Various histological subtypes	Metastases	Naive and pretreated patients	Tumor and peri-tumoral tissue	Immunofluorescence and genes signature	B cell-linage high tumors associated with favorable outcomes	B cell signature and presence of TLS associated with response to immunotherapy.	4
Skin	Various histological subtypes	Primary tumors and metastases	Naive and pretreated patients	Tumor and peri-tumoral tissue	$CD3^+$ and $CD8^+$ T cells, $CD20^+$ B cells, $Ki67^+$ proliferative cells	Favorable	TLS-derived genes signature from pretreatment samples are associated with increased survival in patients treated with CTLA-4 and PD-1 blockade therapies	5
Skin	Various histological subtypes	Primary tumors	Naive	N.A.	HEV detection ($MECA\text{-}79^+$)	High HEV density in lower tumor stages	N.A.	15
Skin	N.A.	Metastases	Naive and pretreated patients	Tumor and peri-tumoral tissue	12 genes signature and immunohistochemistry: $CD3^+$ and $CD8^+$ T cells, $CD20^+$ B cells and $CD86^+$ antigen presenting cells	Favorable	N.A.	50

(*Continued*)

Table 1. (Continued).

Cancer type	Tumor subtype	Tumor localization	Treatment history	TLS localization	TLS composition	Prognostic value	Predictive value	Refs
Skin	Various histological subtypes	Primary tumors	Naive	Tumor and peri-tumoral tissues	$OX40^+$ and $CD25^+$ T cells, $CD1a^+$ DC and mature DC ($DC\text{-}Lamp^+$)	Favorable	N.A.	[44]
Skin	N.A.	Metastases	Pretreated patients	Tumor and peri-tumoral tissues	$CD20^+$ B cells, $CD138^+$ plasma cells, $CD21^+$ FDC, $MECA\text{-}79^+$ and $Pnad^+$ HEV	N.A.	N.A.	[70]

N.A., not available; Ti-BALT, tumor-induced bronchus-associated lymphoid tissue; FDC, follicular dendritic cells; MSI-H, colorectal cancer with high levels of microsatellite instability; MSS, colorectal cancer with low microsatellite instability; DDLPS, dedifferentiated liposarcoma; LMS, leiomyosarcoma; UPS, Undifferentiated pleomorphic sarcoma; GIST, gastrointestinal stromal tumors; EMRS, embryonal rhabdomyosarcomas; AMRS, alveolar rhabdomyosarcomas.

Table 2. The prognostic value of B lymphocytes in cancer.

Cancer type	Tumor subtype	Treatment history	Cell type	Identification markers	Prognostic value	Refs
Bladder cancer	Muscle-invasive bladder cancer	Adjuvant platinium based chemotherapy	B cells	CD19	Favorable	73
	Urothelial bladder cancer			IgA1	Associated with advanced disease	74
Breast cancer	Primary operable invasive ductal breast cancer		B cells	CD20	Neutral	75
					Favorable	76
			Plasma cells	CD138	Negative	75,77
	Invasive breast carcinoma		B cells	CD20	Favorable	78,79
	Primary triple- negative breast cancer		B cells	CD20	Favorable	80,81
	Stage II or III	Neoadjuvant chemotherapy	B cells	CD20	Favorable	82
Colorectal cancer			B cells	CD20	Favorable	83_86
			Plasma cells	CD138	Favorable	83
Esophageal adenocarcinoma	Adenocarcinoma of esophagogastric junction		B cells	CD20	Favorable	87
			Plasma cells	CD138	Favorable	87
			B cells	IgKC	Favorable	88
			Plasma cells	CD138	Favorable	88
		Neoadjuvant chemo-radiotherapy	Plasma cells	IgG4	Favorable when associated with ulcer	89
Gastric cancer			Plasma cells	CD138	Favorable	88
			B cells	CD20	Favorable for disease free interval	55,90
			Plasma cells	IgG4	Negative	91
Hepatocellular carcinoma			B cells	CD20	Favorable when associated with high CD3 + count	92
					Favorable when located in tumor margin	93,94
			Plasma cells	IgA PDL1	Negative, associated with advanced disease	95
Renal cell carcinoma	Renal clear cell carcinoma		B cells	CD20, RNAseq B cell signature	Negative	96_98
	Metastatic	Immune checkpoint therapy		RNAseq B cell signature	Favorable, associated with response to treatment	4
Non-small cell lung cancer	Lung adenocarcinoma		B cells	CD20, RNAseq B cell signature	Favorable	96,98_101
			Plasma cells	CD138	Favorable	99,100,102
				IgG	Favorable	96,*103*
	Squamous cell carcinoma		B cells	RNAseq activated/memory signature	Negative	98,102
				IgG4	Favorable	104
	Large cell carcinoma		Plasma cells		Negative	102
Melanoma			B cells	CD20, RNAseq B cell signature	Favorable	96,*98*,105,*106*
	Stage III/IV	Neoadjuvant treatment with immune checkpoint blockade	B cells	RNAseq B cell signature	Favorable, associated with response to treatment	4
			Plasma cells	CD138	Favorable	105,107
				IgG	Favorable	96,108
				IgA	Negative	108,109
			Plasma cells	IgA	Negative	107
Mesothelioma			B cells	CD20	Favorable	110,111

(*Continued*)

Table 2. (Continued).

Cancer type	Tumor subtype	Treatment history	Cell type	Identification markers	Prognostic value	Refs
Serous ovarian cancer			Plasma cells	CD138	Negative	112
				B cell, IgG signature	Favorable	113
High grade serous ovarian cancer			B cells	CD20	Favorable	114,115
Pancreatic adenocarcinoma			B cells	CD20	Favorable	116,117
			Plasma cells	IgG4	Negative	118
Prostate cancer	Metastatic or therapy-resistant		Plasma cells	IgA	Negative	119
Soft tissue sarcoma			B cells	RNAseq B cell signature	Favorable	3
Squamous cell carcinoma			B cells	CD19	Favorable	120

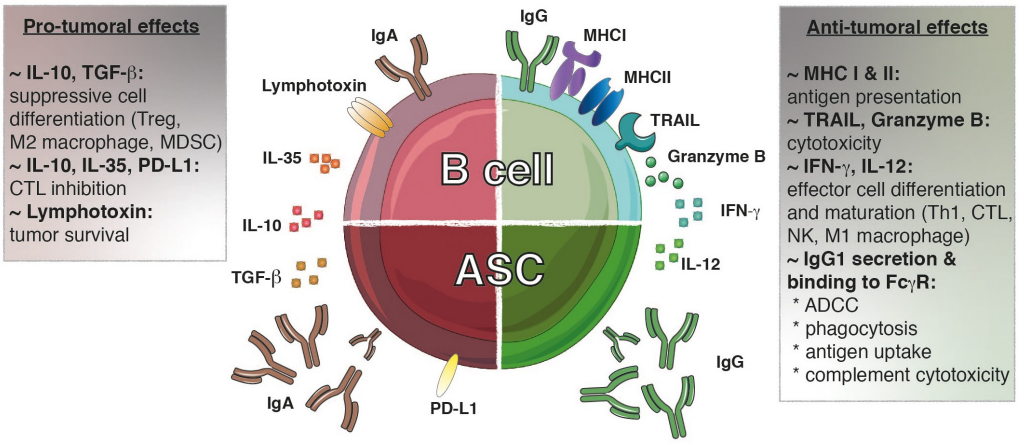

Figure 3. The dichotomy of tumor-infiltrating B lymphocytes. Both pro- and anti-tumoral roles can be attributed to B lymphocytes. IgG1$^+$ B cells promote the anti-tumoral response by presenting antigens to T cells and secreting cytokines (IFN-γ, IL-12) that polarizing the response toward an optimal Th1/CTL composition. These B cells can exert direct cytotoxic functions through the expression of TRAIL and granzyme B. Furthermore, the IgG1 antibodies secreted by the ASC can bind to the FcγR at the surface of NK cells, macrophages and dendritic cells allowing induction of ADCC, phagocytosis and antigen uptake, respectively. Furthermore, IgG1 antibodies fix complement to trigger its cytotoxic cascade. In contrast, IgA$^+$ cells are associated with the secretion of inhibitory cytokines (IL-10, IL-35, TGF-β) that create a suppressive environment favoring the emergence of Treg, M2 macrophages and MDSC while repressing the function of the effector cells. In addition, the expression of lymphotoxin by B cells supports tumor cell survival. ADCC, antibody-dependent cell-mediated cytotoxicity; ASC, antibody secreting cell; CTL, cytotoxic T lymphocyte; FcγR, Fc gamma receptor; Ig, immunoglobulin; IFN-γ, interferon gamma; MDSC, myeloid-derived suppressor cells; MHC: major histocompatibility complex; PD-L1: progammed cell death ligand 1; TGF-β, transforming growth factor-beta; TRAIL, tumor-necrosis-factor related apoptosis-inducing ligand; Treg, regulatory T cell.

complement efficiently, possesses a high affinity to the Fcγ receptors expressed at the surface of myeloid and NK cells, and its binding triggers antibody-dependent cell cytotoxicity (ADCC), phagocytosis and tumor antigen uptake by antigen-presenting cells. Furthermore, IgG$^+$ memory B cells can drive direct cytotoxic activity against tumor cells through the expression of TRAIL or the release of granzyme B[93] (Figure 3). They also secrete IFN-γ, which drives Th1 and cytotoxic response by polarizing macrophages, CD4$^+$ and CD8$^+$ T cells (Figure 3). Hence, IgG$^+$ ASCs and memory B cells make a valuable contribution to the antitumor response, and their presence is positively correlated to increased survival in patients with high-grade serious ovarian cancer,[46,114] hepatocellular carcinoma,[93] melanoma,[105,123] KRAS-mutant lung carcinoma,[103] pancreatic,[124,125] gastric,[64,126] breast[80,113,127] and non-small cell lung cancers.[96,103]

Features of pro-tumor or regulatory B lymphocytes

In contrast to the IgG antibody subclass, both IgA and IgE isotypes have been associated with poor prognosis for patients with hepatocellular carcinoma,[95] melanoma,[107] KRAS-mutant lung carcinoma,[103] prostate[119] and bladder[74] cancers. These isotypes do not fix complement, or mediate the ADCC by NK cells. IgA in particular is a neutralizing antibody that does not trigger inflammatory responses. IgA$^+$ ASCs, expressing suppressive molecules such as progammed cell death ligand 1 (also known as PD-L1) and IL-10, were described in both patient samples and mouse models of hepatocellular and prostate cancer.[95,119] These cells inhibited the cytotoxic T cell response and were involved in resistance to chemotherapy and checkpoint inhibitor therapy. Like regulatory T cells, regulatory B cells can secrete IL-10, transforming growth factor (TGF)-β or IL-35 that will favor switching to IgA and the generation of new suppressive immune cells.[121] In addition, the secretion of lymphotoxin by B cells was shown to favor tumor cell survival in prostate cancers[128] (Figure 3). Although under different designations and defined by various markers, suppressive B lymphocytes have been described in a wide variety of tumor types where they hinder antitumor immune responses. These include hepatocellular carcinoma,[129,130] gastric tumors, [131] squamous cell carcinoma,[132]melanoma[133] and pancreatictumors.[134]

4. Strategies to enhance TLS formation and/or anti-tumor B cell accumulation in cancers

The modulation of TLS or TLS-forming immune cells such as B lymphocytes is an attractive option to (i) induce *de novo* local antitumor immunity in poorly immunogenic tumors, (ii) increase endogenous immune responses, and (iii) redirect a suppressive immune microenvironment toward effective antitumor immunity. Anti-cancer treatments, including chemo- and radio-therapy, or targeted therapies such as immune checkpoint inhibitors, all stimulate the immune system to fight cancer cells. In various cancer types, the presence of TLS has been associated with increased disease free- and overall survival of patients treated with immune checkpoint inhibitors,[4,5,72,135,136] adjuvant trastuzumab (anti-HER2 antibody)[59] or adjuvant and neoadjuvant chemotherapeutic regimens.[81,137,138] These observations indicate that enhancing the formation, and/or maturation, of TLS in tumors could further augment therapy responses and increase the prognosis of cancer patients.

Several approaches have been successful in inducing TLS formation associated with anti-tumor immunity. Vaccination of HPV-driven cervical cancer patients against E6 and E7 proteins[139] or pancreatic carcinoma patients using irradiated allogenic GM-CSF-secreting pancreatic tumor vaccine[140] have been shown to induce TLS formation. In preclinical models, the overexpression of LTα

under the rat insulin promotor in non-tumor bearing mice induced TLS formation in the pancreas and kidneys of transgenic animals.[141] This was exacerbated in the pancreas of mice overexpressing both LTα and LTβ under the rat insulin promotor II[22] and drove T cells, B cells and follicular dendritic cell recruitment which are structurally organized and mimic the configuration of SLO. More recently, the targeting of LIGHT, a member of the TNF superfamily which binds to the lymphotoxin receptor (encoded by *TNFSF14*), to tumor vessels *via* a vascular targeting peptide (LIGHT-VTP) was reported to induce TLS formation and vasculature remodeling in mouse tumors.[142] Importantly, the combination of LIGHT-VTP with immune checkpoint inhibitors increased anti-tumor responses and survival in mice by inducing the recruitment of a large number of effector and memory T cells into tumors.[142] Different approaches were also studied and notably, the injection of dendritic cells engineered to deliver the cytokine IL-36,[143] or the chemokine CCL21,[144–147] were both shown to promote intratumoral TLS formation.

The dichotomy between pro- and anti-tumor immune cell populations such as B and T lymphocytes suggests that patients could be better stratified based on the characterization of TLS-forming cells rather than simply assessing the presence or absence of TLS in tumors. One potential approach is the eradication of the suppressive B cells with an anti-CD20 antibody. However, the use of an anti-CD20 antibody in melanoma increased the development of melanoma metastases in lungs, and the growth of B16 primary melanoma tumors when injected subcutaneously.[148–151] Thus, the tumor context matters, and such strategies need to be evaluated in every tumor type. ASCs, which no longer express CD20 are more complicated to target. Proteasome inhibitors, BAFF/APRIL antagonists (e.g., tacicept or tabalumab) or specific inhibitors of the pro-survival molecules Mcl1/Bcl2 all constitute logical strategies that need to be evaluated. However, for the later, the targeting would also need to be highly specific, depleting suppressive cells and leaving anti-tumor immune subsets including cytotoxic $CD8^+$ T cells or NK cells intact. The use of such approaches could be combined with strategies to impair suppressive functions such as checkpoint inhibitors to target PD-L1. Alternately, it could be combined with engineered bifunctional fusion antibodies such Bintrafusp Alfa (also known as M7824) that simultaneously neutralizes PD-L1 and TGF-β and results in the activation of both innate and adaptive immune systems thereby conferring potent anti-tumor immunity[152] and long-term anti-tumor protection[153] Alternatively, the reduction of accessory suppressive populations, such as regulatory T cells in TLS[154] could divert the microenvironment away from pro-tumor signals leading to tumor eradication.

Future perspectives and outstanding questions

Collectively, TLS and the associated T and B lymphocytes might serve as biomarkers useful to select patients who might better respond to immunotherapy. However, there are still many questions that remain to be answered before they can be incorporated into clinical practice as prognostic tools.[155]

Do immature and mature TLS differentially impact a patient's prognosis?

It is still unclear whether the degree of TLS maturation impacts a patient's prognosis or treatment efficacy. Indeed, whether immature and disorganized TLS with sparse cellular aggregates and no evidence of effective conventional adaptive immunity convey similar prognostic value as mature and structurally well-defined TLS harboring follicles and germinal centers remains unclear. Recently, Li and colleagues (2020) endeavored to examine this issue in oral squamous cell carcinoma. They found that the presence of TLS was associated with increased 5 years overall- and relapse-free survival, and importantly, both immature and mature TLS conveyed equally positive outcomes.[58] In contrast, Posch *et al.* (2018) delineated that TLS in colorectal tumors exhibited different degrees of maturation which were associated with differential prognostic values.[47] In particular, mature TLS containing germinal centers had a more positive prognostic outcome compared with immature TLS.[47] Thus, evaluation of TLS maturation status in every tumor type would bring TLS into focus as an accurate prognostic tool for cancer treatment.

Can patient survival and response to treatment be predicted based on prospective evaluation of TLS ?

In most cases, the presence of TLS in tumors and their correlation with patient outcomes have been evaluated retrospectively. Given the consistent positive correlation of TLS with the anti-tumor immune response, prognosis and immunotherapeutic responses, prospective studies are warranted to determine the utility of measuring TLS presence, composition and density as prognostic tools or predictive markers of therapy efficacy.

Does TLS composition differently impact patient prognosis?

While the presence of TLS often positively impacts clinical outcomes, TLS composition itself might dictate treatment efficacy, tumor recurrence and patient survival. Indeed, Yamaguchi and colleagues[156] classified TLS into five categories based on their immune cell composition and found that TLS enriched in helper T cells were associated with disease relapse in advanced colorectal cancer. Another example is the diversity found among ASC where IgA producing cells are almost exclusively associated with a poor prognosis, while IgG^+ secreting cells frequently correlated with increased patient survival. Thus, better understanding of the composition of the immune infiltrate and function of TLS-forming cells, such as the isotype of ASC may be important.

Therapeutic intervention – can we specifically induce or enhance TLS formation in tumors?

Strategies augmenting *de novo* TLS formation in patient tumors could potentiate antitumor treatments leading to an increase in

therapy response rates and patient progression- and overall-survival. While a number of preclinical studies have demonstrated the potential value of such treatments, additional studies and clinical trials are necessary to determine the therapeutic value conveyed by combining TLS- or B lymphocyte-specific targeting with current immunotherapeutic treatments.

The development of new technologies that enable the interrogation of more than 50 cellular markers simultaneously allows the precise characterization of the cellular composition, function and localization within tumors.[157–159] Similar approaches could be used to investigate in detail TLS composition and function. Recently, Schurch and colleagues[160] elegantly identified nine conserved distinct components characteristic of colorectal cancer immune microenvironment – described as 'cellular neighbourhoods' – which differentially impact a patient's survival. The expansion of our capabilities to study a large number of parameters simultaneously might increase our understanding of the tumor microenvironment. This is likely to shed light on the cellular and molecular events associated with TLS formation and intratumoral T and B lymphocytes function associated with successful anti-tumor immunity and therapy responses.

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Disclosure of interests

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Disclosure statement

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