Tertiary lymphoid structures in head and neck squamous cell carcinoma (Review)

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Abstract. The tumor microenvironment in head and neck squamous cell carcinoma (HNSCC) has a critical role in tumor progression and prognosis. The organization of T cells, B cells and high-endothelial venules into structures termed tertiary lymphoid structures (TLS), are associated with favorable outcomes in HNSCC. Despite their importance, the formation and function of TLS-associated immune cells in HNSCC have remained elusive. The aim of the present review is to elucidate the mechanism underlying TLS formation, which may contribute to the development of novel and effective immunotherapies for HNSCC, and summarize recent advances in HNSCC treatment.

Contents

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1. Introduction

Head and neck cancer is the sixth most common malignancy worldwide (1). It is a highly aggressive solid tumor and patients with this malignancy have low five-year survival rates, at <50% (2,3). The activation of cancer-related signaling pathways leads to genetic and epigenetic alterations, and this leads to the development of malignancies, including head and neck squamous cell carcinoma (HNSCC) (4). HNSCC surveillance against development and progression markedly depends on the tumor microenvironment (TME) and its interactions with the tumor.

The TME has been recently reported to play a pivotal role in sustaining tumor cell survival and protecting them from immunotherapy and chemotherapy-induced death. The TME in HNSCC consists of a variety of cell types infiltrating the tumor and interacting with tumor cells and with one another to promote tumor progression or mediate antitumor immune responses (5). Compared with T cells, B cells have been investigated to a much lesser extent (6). However, current studies have substantiated the function of B cells as an important prognostic factor for patients with HNSCC. B cells have been found to be associated with the formation of tertiary lymphoid structures (TLS), promoting immunotherapy response (7). However, the function of TLS in HNSCC has remained elusive.

Increasing attention has been paid to TLS, which are ectopic lymphoid tissues (8). TLS formation is dependent on chronic inflammation at any site in response to infection, autoimmune responses or the TME (9,10). TLS are characterized by mature dendritic cells (DCs) in the T-cell region adjacent to B-cell follicles within germinal centers (GCs) (11,12) (Fig. 1). The GCs within TLS play a crucial role in the immune response and disease progression. The primary molecules implicated in GCs of TLS, each playing a unique role in maintaining the GC environment and supporting immune functions, such as CD21, CD23, inducible T cell costimulator (ICOS), C-X-C motif chemokine ligand

(CXCL)10, IFN-γ, TNF, caspase (CASP)8, CXCL13, activation induced cytidine deaminase (AICDA) and lymphotoxins. For instance, CD21 and CD23 are important markers for detecting GC cells. CD21 helps capture and present antigens to B cells, while CD23 aids in antigen processing and presentation. ICOS is crucial for the development and function of GCs, as it promotes the differentiation of T-follicular helper cells, which support B-cell activation and antibody production (13). CXCL10, a chemokine, attracts immune cells to the GC, helping to form and maintain its structure (14). IFN-γ, a cytokine, enhances immune cell functions and regulates the overall immune response within the GC. TNF, another cytokine, drives inflammation and supports the survival and activation of immune cells (15). CASP8, involved in apoptosis, helps balance cell proliferation and death within the GC. CXCL13 is essential for recruiting B and T cells to the GC, while AICDA drives the production of high-affinity antibodies through processes like somatic hypermutation and class-switch recombination. Finally, lymphotoxins like LTα3 (a soluble homotrimeric cytokine that binds to TNF receptors) and LTα1β2 (a membrane-bound heterotrimeric protein composed of LTα and LTβ subunits) help shape and preserve the GC structure by influencing stromal cells and follicular dendritic cells (14). Together, these molecules work in harmony to ensure the proper functioning of GCs, supporting the adaptive immune response and influencing disease outcomes. High endothelial venules (HEVs) are specialized post-capillary venules found in secondary lymphoid organs and tissues, such as lymph nodes, tonsils, Peyer's patches, and other lymphoid aggregates. Together with DCs play pivotal roles in effector function, affinity maturation and antibody generation, which are associated with positive clinical outcomes, upon administration of systemic antitumor immunotherapy, particularly in solid malignant tumors such as HNSCC (16).

Recent studies have demonstrated that TLS presence positively correlates with the prognosis in most solid tumors (17-20). In non-small cell lung cancer (NSCLC) (21), the presence of TLS correlates with improved patient outcomes, with high-density TLS linked to enhanced survival rates and augmented efficacy of anti-programmed cell death ligand 1 (PD-L1) therapies. Regarding triple-negative breast cancer (22), elevated levels of CCL19+ DCs and CXCL13+ T cells are associated with better prognoses. In uveal melanoma (23), the presence of TLS prolongs progression-free and overall survival (OS). In colorectal cancer (24), high lymphocyte infiltration, density and maturation within TLS are associated with favorable patient outcomes. In breast cancer (25), high-density TLS are linked to improved prognoses, with CCL21+ and apolipoprotein D+ fibroblasts playing a crucial role in TLS formation. In HNSCC, specific T-cell subsets, such as programmed cell death 1 (PD1)+CXCL13+CD8+ T cells, have been identified within TLS. These cells exhibit robust antitumor capabilities. The presence of such cell subsets further underscores the unique role of TLS in HNSCC, which may not be as prominently observed in other cancer types (26).

Of note, essential TLS mechanisms in adaptive immunotherapy, particularly for HNSCC, are under investigation. Although existing studies have confirmed the significance of tumor-associated TLS in HNSCC (27), current investigations have primarily focused on their biological functions and prognostic value. In contrast to previous research directions, the present review aimed to highlight and summarize the current knowledge on cellular and molecular biological requirements for TLS formation and the role and impact of B cells and TLS in antitumor immune response in HNSCC, which may contribute to the development of promising TLS-focused immunotherapies. This review systematically presents a comprehensive analysis of cutting-edge developments in TLS-targeted immunotherapeutic approaches, encompassing both novel TLS induction techniques and combination therapies that leverage TLS formation to potentiate immune responses. These emerging strategies demonstrate significant potential for improving therapeutic outcomes in HNSCC management. The present review provides a theoretical basis for the clinical diagnostic value and antitumor therapeutic efficacy of TLS in HNSCC.

2. Formation and signature of TLS in HNSCC

Most TLS are typically organized, with distinct T- and B-cell regions and homeostatic chemokines, including C-C motif chemokine ligand (CCL)2, CCL3, CCL4, CCL5, CCL18, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13, CXCR4 and lysosomal associated membrane protein 3 (LAMP3), which organize secondary lymphoid organ (SLO) microarchitecture (28-32). Similar to SLO formation, TLS formation primarily requires local aggregation of receptor activator for nuclear factor κB ligand (RANKL), CXCL13, and interleukin (IL)-17 induced by lymphoid tissue inducer (LTi) cells, which are specific immune cells that accumulate at the site of inflammation (32,33). LTα1β2, IL-17 and RANKL expression in LTi cells interacts with LTβR expression in mesenchymal lymphoid tissue organizer cells and plays a pivotal role in chemokine release (Fig. 2) (34-36). The expression of some of these factors is further amplified by interactions with other cell types, such as natural killer (NK) cells and DCs, culminating in the production of pro-angiogenic molecules (vascular endothelial growth factors A and C) and recruitment of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (11,33). The absence of endothelial Notch signaling has been shown to promote the formation of TLS. This suggests that the Notch signaling pathway in endothelial cells plays a crucial role in regulating TLS development, and its inhibition may create an environment conducive to TLS formation (37).

HEVs are post-capillary veins composed of cubic blood endothelial cells that release sulfated sialomucins, which bind to lymph nodes and lymphoid tissue. HEV colocalizes with TLS in tumors, thereby promoting initial immune cell recruitment and activating immune cells from TLS into the circulation (11,34). HEV transfers naive lymphocytes from the blood to the lymph nodes via morphologically and functionally specialized blood vessels, and in HNSCC, lymphocytes are initiated by antigen-presenting cells (38). In addition, the peripheral node addressin $(PNAd)^+$ HEVs are an indispensable HEV subgroup essential for the identification and prognosis of HNSCC, around TLS (39). Recently, a subtype of TLS known as 'follicle-like TLS (FL-TLS)' has been identified as an independent prognostic marker in laryngeal cancer. Patients

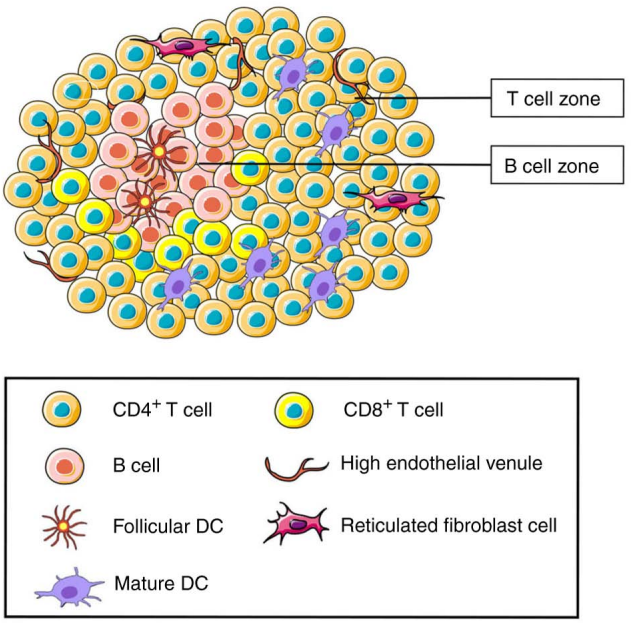

Figure 1. Structural composition of TLS. The structural composition of TLS, including T cell zones (populated by T lymphocytes, primarily CD4+ and CD8+ T cells), B cell zones (contain B lymphocytes, often organized into follicles) and FDCs (located in the B cell zone of TLS, where they closely interact with B cells, providing both physical and signaling support to B cells), mature DCs (distributed in the T cell zone of TLS and may also be found in the peripheral regions of TLS, where they participate in recruiting and activating other immune cells). TLS, tertiary lymphoid structures; FDCs, follicular dendritic cells.

with FL-TLS exhibit increased immune cell infiltration and demonstrate enhanced responses to immunotherapy (17). To facilitate the clinical translation of TLS, efforts are underway to establish standardized evaluation protocols, incorporating advanced tools such as artificial intelligence and spatial omics for precise analysis.

Although the exact mechanism of autonomous TLS formation in humans remains controversial, it is primarily involved in the adaptive immune response, and TLS presence may result in satisfactory clinical outcomes and prolong disease-free survival (DFS) of patients with cancer. A recent study has found that TLS are positively associated with survival in both patients with human papillomavirus (HPV^+) and HPV^- HNSCC (6,40). Multiple analyses have revealed that T cells, $CD20^+$ B cells, $PNAd^+$ HEVs and DC-$LAMP^+$ (LAMP3) DCs are present in both mature and immature TLS in HNSCC (41,42). Intratumoral B cells were predominantly observed in TLS and were associated with progenitor exhausted $CD8^+$ T cells and enhanced PD-1 blockade treatment. In addition, semaphorin 4A, a membrane-bound glycoprotein essential for the type 2 T-helper cell (Th2) response in humans, regulates HNSCC TLS formation in patients, as it is closely associated with immune aggregate generation (43,44). The maturity of TLS can be effectively assessed using hematoxylin and eosin staining, a standard histological technique. This assessment is crucial because the maturity of TLS plays a significant role in the overall response to neoadjuvant treatment. Understanding the relationship between TLS maturity and treatment outcomes could provide valuable insights for optimizing therapeutic strategies (45).

3. TLS-associated immune cells in HNSCC

The TME is characterized by its complexity and heterogeneity. Tumor-infiltrating lymphocytes (TILs) play a significant role in patient heterogeneity and clinical outcomes (46). Researchers have established that immune cells within a tumor can organize into TLS (47). This review focused on the critical role of immune cells clustered around tumors in TLS development and function.

B lymphocytes. The pivotal role of B cells in antitumor immune responses, particularly following the onset of malignant tumors, has been increasingly reported. Notably, a better prognosis is associated with high densities of $CD20^+$ B cells in the TME of head and neck cancer (48). Furthermore, a significant heterogeneity in the maturation status and effector function of B cells is observed in tumor-infiltrating TLS. In addition to producing antibodies and presenting antigens, B cells play a role in peritumoral immunity by interacting with T cells to form TLS (49,50). In addition, B cells influence other immune and non-immune cell subsets in the TME by producing various cytokines and cellular mediators.

The B-cell population is heterogeneous with different phenotypic and functional subpopulations. Therefore, tumor-infiltrating B-cell (TIL-B) phenotypes are crucial in developing B cell-related immunotherapies for patients with cancers (51,52). As reported by Bruno *et al* (53), TIL-Bs can present antigens to CD4 T cells, suggesting their potential to promote antitumor immunity. Furthermore, memory B-cell clusters [immunoglobulin (Ig) G-$CD38^-IgD^-CD27^+$] are primarily responsible for responding to secondary stimuli and differentiating into antibody-secreting cells in patients with HNSCC.

Furthermore, it has been demonstrated that TLS are associated with a favorable prognosis due to the development of B cell-dependent antitumor immunity. Unlike the B cells in HPV- tumors, the B cells in HPV+ HNSCC tumors exhibit the centroblast phenotype, which is characteristic of GC B cells from tonsils and putative interactions between B and $CD4^+$ TFH cells (54). In a study by Lechner *et al* (55), TLS at different developmental stages were detected in the tumor stroma of HNSCC, mainly at the invasive margins, by staining $CD20^+$ B cells. GCs facilitate the generation of high-affinity antibodies by B cells via mechanisms such as somatic hypermutation and class-switch recombination. Studies indicate that B cells in TLS are directed by the chemokines CXCL13 and CXCL12, and their differentiation into plasma cells is orchestrated through interactions with follicular dendritic cells (FDCs) and follicular helper T cells (Tfh). This indicates that B-cell differentiation is associated with the maturation of germinal centers (17). A study demonstrated a promising potential to establish an association between B-cell marker genes (BCMG) and TLS in relation to patient responses to ICIs (54). This association is particularly significant because it suggests that the presence and activity of BCMG and TLS could provide valuable insights into how well patients may respond to ICI therapy. Furthermore, this relationship may also serve as an important predictor of OS in patients, indicating that BCMG and TLS could be utilized as biomarkers to guide treatment decisions and improve patient outcomes in cancer care (56). In addition, high numbers of plasma cells (multiple myeloma 1)

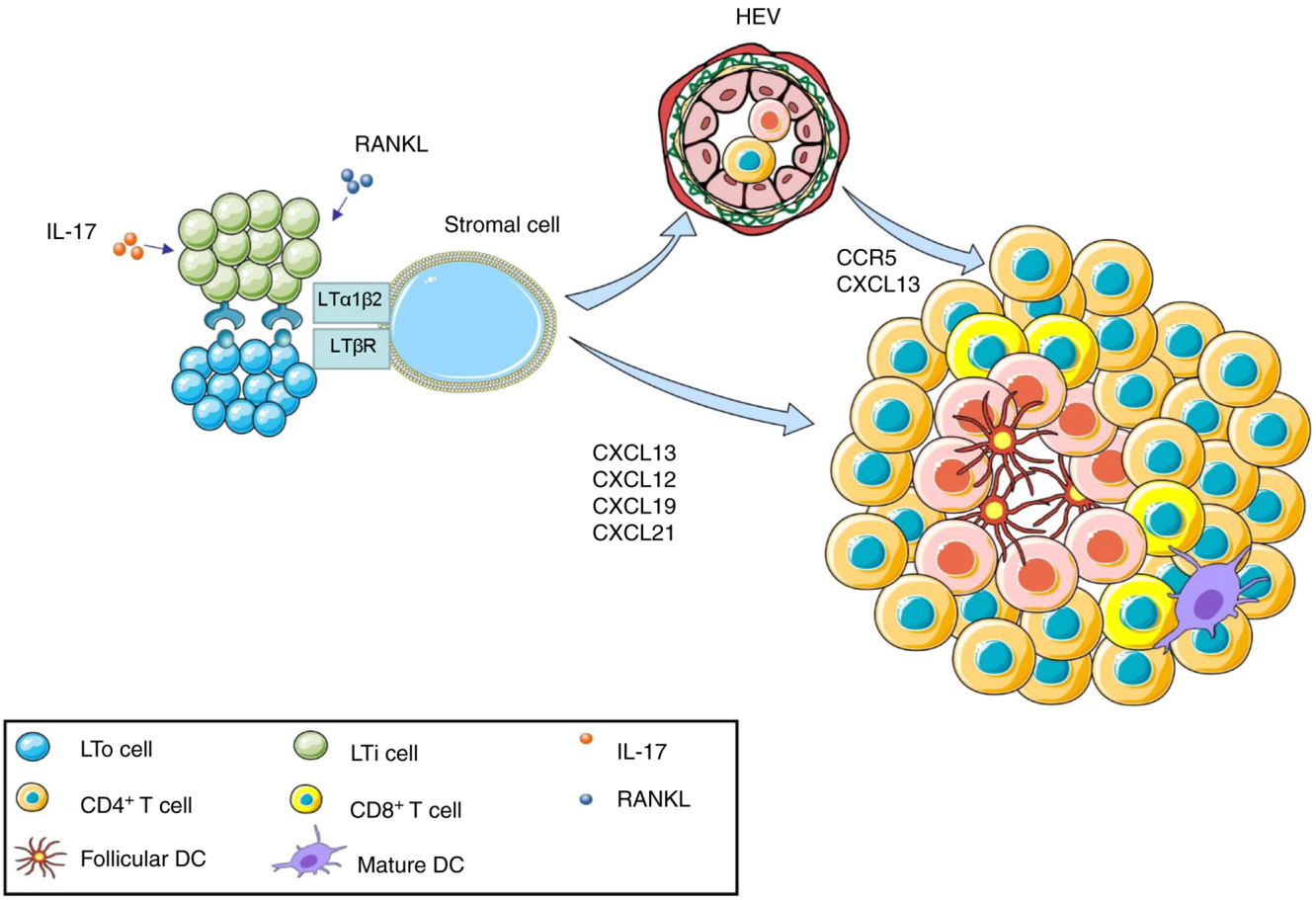

Figure 2. Genesis of tertiary lymphoid structures. The cytokine IL-7 recruits LTi cells to sites of inflammation. These LTi cells interact with local stromal cells, primarily through the binding of LTα1β2 to the LTβR. The combined effects of LTβ-LTβR signaling and IL-17 secretion by LTi cells recruit lymphocytes from nearby HEVs into T cell and B cell zones or stimulate the production of key chemokines, including CXCL12, CXCL13, CCL19 and CCL21, orchestrating their organization into tertiary lymphoid structures. LTi, lymphoid tissue inducer; LTo, lymphoid tissue organizer; LTα1β2, lymphotoxin-α1β2; LTβR, LTβ receptor; HEVs, high endothelial venules; RANKL, RANK, receptor activator of nuclear factor kappa-B ligand; CXCL12, C-X-C motif chemokine ligand 12.

were detected around and in TLS and TFh cells, which are essential for B-cell maturation and survival in GCs. Plasma cells are generated as a result of mature FDCs selecting B cells with high-affinity B-cell receptors and, in collaboration with Tfh, inducing further maturation and differentiation of these B cells (17). FDCs are a key cell type in TLS, are derived from mesenchymal cells, are primarily located in the follicular regions of TLS and can typically be identified by markers such as CD21 and CD23 (13). FDCs, in conjunction with other stromal cells, create a dense stromal network akin to the one formed by follicular reticular cells in SLOs (57). This intricate network serves to stabilize the structure of TLS and securely anchor them within tissues impacted by chronic inflammation.

Multiple studies have demonstrated that the activated B cells in TLS present tumor-derived peptides that may modulate T-cell phenotypes in the TME (58,59). Accumulating evidence suggests that the activated B cells in HNSCC may express CD40, CD80 and ICAM-1 and present antigens to CD4 T cells (6,60). Researchers need to further confirm and investigate the precise roles of B cells in TLS and the associated processes.

T cells and NK cells. Of note, TLS-enriched tumors are more likely to be infiltrated by $CD8^+$ T cells, which may express suppressive immune checkpoints after persistent antigen exposure. The co-occurrence of $CD8^+$ T and $CD57^+$ NK cells in TLS seems to confer a better patient prognosis (40). TLS have been shown to enhance antitumor effects by recruiting CD8 T cells in a hormonal model of tongue tumors in mice with HNSCC (61). The DCs and T cells activated in TLS can facilitate NK-cell proliferation by producing various cytokines such as IL-2, IL-5 and IL-8 (62). In addition, chemokines such as XCL2 play a critical role in stimulating DC recruitment into the TME, which is important for TLS maturation and invasion in laryngeal squamous cell carcinoma (45). Other chemokines, such as CXCL8 and CX3CL1, can also contribute to the recruitment of NK cells to tumor sites (39). These findings suggest that high levels of $CD8^+$ T and NK cells in patients with oral squamous cell carcinoma (OSCC) are significantly associated with TLS grading However, a study by Trüb and Zippelius (63) demonstrated that gene and protein expression in T cells varies significantly based on T-cell location relative to TLS. Compared with the T cells isolated from tumors without TLS, the T cells isolated from TLS show increased expression of activated markers, such as Tcf1/Tcf7 mRNA, involved in stem-like T-cell population maintenance, as well as IL-7R and selectin L, which encode CD62L, and decreased expression of transcripts for granzyme-encoding

genes and protein tyrosine phosphatase non-receptor type 22, a negative regulator of T-cell receptor signaling (64,65). Interestingly, the Tcf1/Tcf7^{+} T cells positively correlate with the number of TLS in OSCC. TLS with an increased Tcf1/Tcf7 frequency in T cells are associated with a better prognosis (66). These findings may open new avenues for therapeutic strategies aimed at increasing TLS formation and enhancing TLS function.

Treg cells, a subset of T cells, typically inhibit antitumor immunity and play a significant role in tumor immunotherapies. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is expressed by Treg cells and controlled by forkhead box P3 (Foxp3), which inhibits the activation of conventional T cells by downregulating CD80/86 expression in antigen-presenting cells (67). In precancerous lesions of head and neck cancers, the upregulation of Treg cell infiltration correlates with malignancy. Treg cells are also detected in tumor-associated TLS, and Foxp3+ Treg cell infiltration positively correlates with TLS in oral epithelial dysplasia, a precancerous lesion of oral cancer (68). Increased infiltration or differentiation of Treg cells may promote the development of oral epithelial dysplasia (68). In addition, TLS are more effective in Treg cell recruitment than killer T cells are, which suggests that TLS are more likely associated with the suppression of T-cell killing. Recent findings indicate that ATP binding cassette subfamily B member 11 gene accumulates in immature TLS, which further enhances the infiltration of immunosuppressive Tregs and induces resistance to PD-1/PD-L1 inhibitors in HNSCC (69). These findings indicate that TLS may increase the risk of cancer by increasing Treg cell infiltration.

Furthermore, TLS-associated B cells in the TME favor intratumoral CD4+ T-cell expansion (70). Similar to CD8+ T cells, CD4+ T cells show CD8+ T-cell responses of the Th1, Th2, Th17 and TFh lineages (63,71). The antitumor response of Th1 cells is associated with TLS B cells, which play a vital antitumor role by producing IFN-γ and recruiting NK cells (72). Studies on patients with OSCC suggest that patients with high levels of CD57+ NK and T cells have high OS rates (47). NK cells in carcinomas are recruited via the chemokines CXCL8 and CX3CL1, and the proliferation of NK cells can be enhanced by cytokines, including IL-1, IL-12, IL-14 and IL-18, produced by T cells (72-74). However, the complex functions of the subsets remain elusive and require further investigation.

DCs. During tumor progression, DCs migrate from the tumor site to lymphatic vessels to form TLS. TLS may allow T cells to target tumor-associated antigens that are serially sampled from immature DCs and then treated via mature DCs in TLS (74).

Dieu-Nosjean *et al* (75) found that mature DCs and TLS are required for an efficient CD8+ T-cell immune response, as increased DC density correlates with higher CD8+ T-cell density in colorectal cancer. Furthermore, Giraldo *et al* (74) found that TLS-associated DCs negatively correlate with the number of PD-1+ infiltrating T cells, suggesting that the presence of TLS and mature DCs may be related to diminished T-cell exhaustion in cancers. Further investigation should focus on the function and mechanism of DCs in TLS immune reactivity.

Tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). TAMs and MDSCs, which infiltrate the TME, play two major roles in creating tumor immunosuppressive microenvironments. The effects of TAMs on tumor progression and TAM response to anti-cancer therapy critically correlate with apoptosis, new vessel formation and inflammation (76). The association between MDSC accumulation and tumor formation in oral cancer has been confirmed through extensive research (77).

However, only a limited number of studies have reported the number of TAMs and MDSCs in TLS. Notably, CX3CR1hi macrophages can induce TLS in the gut via an IgA response (78). Furthermore, MDSCs in TLS negatively affect the ability of TLS to produce effector cells and memory lymphocytes (79). Indeed, the functions of TAMs and MDSCs in the TLS in HNSCC are inconclusive and lack consensus. In the future, investigating the mechanisms of TAMs and MDSCs in TLS and their effects on tumor progression will likely be of interest to researchers.

4. Prognostic value of TLS in HNSCC

The prognostic value of TLS has been widely evaluated. In particular, several clinical trials suggest that TLS may serve as novel prognostic biomarkers and predictors of response to immunotherapy (80). Certain studies have evaluated various markers, such as CD20, CD20/CD21, DC-LAMP, Ki67 and BCL6, to determine the presence of TLS. The presence of CD20, the proliferation marker Ki67, transcription factor BCL6 and activation-induced deaminase in B cells correlate with the prognoses of NSCLC and ovarian cancers (81). Furthermore, previous studies have found that HEVs, which are specific blood vessels in lymph nodes, can recruit B and T cells from the blood to lymphoid tissues, which may help eliminate tumors (80,82,83). HEVs located at the periphery of TLS are characterized by the markers PNAd and the vascular addressin MECA-79 (recognizes a carbohydrate epitope present on a group of sulfated sialomucins) (81). PNAd+ HEVs are significantly associated with the prognosis of patients with OSCC and are critical for the proper identification of TLS in OSCC (41). Furthermore, in breast cancer, HEVs appear to contribute to tumor suppression by promoting high TIL levels (84). Martinet *et al* (83) found that HEVs surrounded by fascin+ and DC-LAMP+ DCs in breast tumor stroma and the density of DC-LAMP+ DC clusters strongly correlate with the density of tumor HEVs, T- and B-cell infiltration, and clinical outcomes for patients. In addition, GCs in TLS strongly correlate with increased survival in patients with HPV^{+} HNSCC (10,85). Of note, patients with HPV+ HNSCC with a higher disease burden have fewer TLS in primary tumors, whereas high TLS levels in tumors are found in patients with a lower disease burden. This suggests the potential of TLS to predict primary tumor recurrence (86,87), which highlights the benefits of immunotherapy for patients in need.

5. Therapeutic manipulation of TLS

Induction of TLS neogenesis. Induction of TLS plays a promising role in immunotherapy in patients with cancer (79). One study mapped TLS-associated chemokine receptors and, based

Table I. Developing strategies for TLS induction in immunotherapy.

Item	Location of expression	Treatment	Effect	(Refs.)
TLS neogenesis				
Lymphotoxin α	Primarily on T-, B-, NK and LTi cells	Pateclizumab	Influence on TLS formation	(12)
Lymphotoxin β receptor	Primarily on T-, B-, NK and LTi cells	Baminercept	Influence on TLS formation	(12)
RANK/RANK-L	Primarily on LTi cells	Denosumab	Influence on TLS formation	(12)
LIGHT	Broadly expressed			
B-cell region				
ICOS/ICOS-L	Primarily active T cells	AMG 557	Inhibition of Treg proliferation	(113,114)

LIGHT, tumor necrosis factor superfamily member 14; NK, natural killer; TLS, tertiary lymphoid structures; RANK, receptor activator of nuclear factor kappa-B; RANK-L, Treg, T-regulatory cell; RANK ligand; ICOS, inducible T-cell costimulator; ICOS-L, inducible T-cell costimulator ligand.

on functional and evolutionary trajectories, proposed the induction of *de novo* TLS in HNSCC to enhance antitumor immune responses by reconstructing TLS immune structures (88-92). Several approaches have successfully induced TLS formation and antitumor immunity (28,93). For instance, CCL21 and LT-βR are located at the center of peripheral lymphoid organs during TLS development (50). A current phase I clinical trial is focused on lymphoid chemokines, which represent main therapeutic targets for TLS regulation (94). During lymphocyte formation, LT-βR signaling induces chemokines, such as CCL21/CCR7 and CXCL13/CXCR5, owing to their associated host protection (Fig. 2) (8). Furthermore, LTβR and its two ligands, LTα1β2 and TNF superfamily member 14 (LIGHT), are expressed in tumor cells in various cancers (95). A study has revealed that the antibody against the LT-βR agonist CBE11 (LT-βR-activation) enhances lymphocyte infiltration in cancer, inhibits tumor growth and induces tumor necrosis (83). Therefore, in addition to a straightforward antitumor effect, LT-βR agonists may promote the generation of TLS, which play a critical role in the development of antitumor immune responses.

A mouse model experiment has demonstrated that recombinant antibodies targeting LTα to tumor cells induce intertumoral HEVs and B- and T-cell activation, which is positively associated with survival (85,95). In a recent study, a dual-adjuvant nanovaccine was found to activate the LT-α and LT-β pathways, which enhanced chemokine expression in the TME, promoted the formation of TLS and inhibited the progression of simian nasopharyngeal carcinoma (96). Of note, HEVs are tightly co-localized with TLS, which suggests that these blood vessels are the primary pathway for circulating immune cell recruitment in active lymphocyte areas (82). Of note, the induction of HEV formation could be an attractive strategy for enhancing lymphocyte recruitment in TLS; thus, exploring the underlying mechanisms of therapeutic TLS formation is necessary.

TLS in immunotherapy. ICI therapies, including adjuvant cemiplimab, PD-1 and CTLA-4, and neoadjuvant chemotherapeutic regimens, are promising for patients with HNSCC. However, low response rate is a major limitation of ICI therapies (97). Multiple factors, including the TME, could influence the efficacy of ICI therapy, and certain biomarkers may predict patient prognosis (98). A recent study involving surgical samples with NSCLC revealed that B-cell infiltration and retention within TLS significantly enhance patients' responses to ICI (99). Additionally, ICI therapy is associated with neoadjuvant-associated tissue reactions, activation of TLS at the tumor bed and off-target immune-related adverse events (100). Currently, most publications support the importance of TLS in antitumor responses, as TLS represent the primary site of antitumor immune response (34). TLS in various cancers are associated with improved DFS and OS rates of patients treated with ICIs (101-103). These observations indicate that enhancing the maturation and formation of TLS with therapeutic interventions may have a predictive function (104). The proximity of TLS to tumor cells was found to be associated with the degree of response to ICIs in patients with HNSCC (105). Various studies have aimed to develop strategies to induce TLS and enhance immunotherapy (74,106-108) (Table I).

Furthermore, previous studies have demonstrated that ICI therapy can produce TLS or organized lymphoid aggregates in the TME, which is highly associated with treatment response and survival benefits in several solid tumors, including bladder, hepatocellular, breast and lung cancers (109-111). Sweeney *et al* (112) reported that following ICI therapy, TLS exhibited a perivascular distribution of cellular lymphocytic and plasma cell infiltrates in the tumor bed. Furthermore, histopathological, immunohistochemical and molecular features revealed that upon ICI treatment, TLS displayed a grenz zone and dermal lymphoid and plasma cell infiltrates with GCs (112). Based on these findings, ICI therapy may initiate a coordinated antitumor response via TLS. A Phase 1 clinical trial is currently underway to evaluate Ad5/3-E2F-D24-hTNFa-IRES-hIL-2 both as a monotherapy and in combination with TIL therapy in patients with solid tumors and metastatic melanoma (NCT04695327 and NCT04217473, respectively) (113).

Lewis' lung cancer experiment revealed that PD-1 and CTLA-4, targeted with LIGHT-vascular targeting peptide to sensitize pancreatic cancer, could partially induce TLS (91). Furthermore, a study on high-grade cervical intraepithelial neoplasia demonstrated that vaccination targeting HPV E6 and E7 proteins induces the formation of TLS containing

Table II. TLS-based antitumor immunotherapy treatment methods for head and neck squamous cell carcinoma.

Method	Examples	(Refs.)
Inducing or reconstructing TLS		
Biomaterials and tissue engineering	aAVCs deliver immune-stimulating signals for artificial adjuvant vector cell therapy. As a novel composite scaffold, using a sol-gel technique to combine bioglass 58 S with a poly L-lactic acid.	(91)
	Biocompatible scaffold materials, mimicking the natural extracellular matrix or lymphoid tissue structure to support the formation and function of TLS. As the clinical trials NCT01997437, NCT02949414.	(92)
Induction of TLS neogenesis		
Biomaterials	Gold nanoparticles conjugated to anti-PD-L1 antibodies	(79)
	A single intratumoral STINGel injection loaded with CDN ML RR-S2 CDA. ML RR-S2 CDA is a synthetic cyclic dinucleotide (CDN) containing an atypical 2'-5' phosphodiester bond, is an activator of the stimulator of interferon genes (STING).	(80)
	L-NIL, a potent small-molecule inhibitor of inducible nitric oxide synthase	(81)
Targeting TLS		
Delivering cytokines	A 'layer-by-layer' nanoparticle system	(98)
	A biodegradable porous silicon nanoparticle	(99)
	A single intratumoral injection of biodegradable polylactic acid microspheres loaded (100), such as polylactic-co-glycolic acid monomers containing 12-carbon.	
	Biodegradable polylactic acid microspheres	(101)
	Co-formulations of cytokines with chitosan solutions	(102)
	An mRNA lipid nanoparticle	(103)

TLS, tertiary lymphoid structures; aAVCs, artificial adjuvant vector cells; anti-PD-L1, anti-programmed death-ligand 1; CDN, cyclic dinucleotide; STING, stimulator of interferon genes.

antigen-experienced effector memory T cells (114). This information provides a novel foundation for future research on antitumor therapy.

A positive relationship between PD-1$^+$ T cells and tumor-associated TLS and on patient survival has also been demonstrated in a novel study. PD-1$^+$ T cells mediate the recruitment of immune cells to TLS by producing CXCL13 (115). The presence of PD-1 cells is a striking predictor of survival in HNSCC treated with PD-1 blockade (116). Studies have shown that TLS enhance the response to PD-1 blockade therapy in a mouse model of HPV-associated HNSCC. These findings suggest that inducing TLS formation in HPV HNSCC tumors could be a promising therapeutic strategy to increase the response rate to immune checkpoint blockade in patients with this disease (40). Considering the characteristics of tumor-reactive PD-1 bright cells in cancers, a potential interventional treatment could be developed. Furthermore, numerous drug combination trials using immune checkpoint blockade therapies combined with TLS induction have been conducted. Investigating syngeneic models of treatment-naïve oral cancer revealed that high immunogenicity (MOC1) models are sensitive to ICIs, while low immunogenicity (MOC2) models exhibit primary resistance to ICIs. In the study, improved tumor response and survival rates were observed when ICIs (anti-PD-L1 or anti-PD-1) were combined with Ad5-CMV-mTNFα/mIL-2, the mouse version of TILT-123 (113). It is expected that future investigations will lead to the identification of an effective immune checkpoint for better and more precise HNSCC treatment.

6. Conclusion

Evidence indicates that TLS are essential for the development of protective T- and B-cell immunity against cancer. In particular, TLS density is associated with patient survival in numerous solid tumors and inversely correlated with the severity of graft rejection and autoimmune diseases, which highlights the positive effect of TLS. Therefore, TLS are potential markers of effective antitumor immunotherapy.

TLS composition is similar to that of SLOs, which includes a B-cell region beset by a T-cell region. In HNSCC, CD3$^+$ T and CD20$^+$ B cells, PNAd$^+$ HEVs and DC-LAMP$^+$ (LAMP3) are common components of mature and immature TLS (6). TLS-associated immune cells are critical for TLS function and antitumor immune responses. In particular, TLS comprise T-cell, B-cell and DC subsets, and their functions and relationship with HNSCC were presented in this review.

The prognostic value of TLS in cancers has been increasingly reported. Furthermore, TLS in tumors are potential targets

for ICI treatment, such as treatment with PD-1, which induces $CD8^+$ T-cell infiltration in HNSCC. TLS neogenesis is induced by chemokines, such as CCL21/CCR7 and CXCL13/CXCR5, owing to their role in host protection (Fig. 2). Furthermore, novel immunotherapies include antibodies targeting HEV structures or chemokines involved in the regulation of TLS formation (Table II).

TLS face challenges in clinical applications due to limitations in detection methods, heterogeneity and complex induction processes (117). Traditional detection methods are invasive and prone to errors, while TLS heterogeneity complicates their clinical utility. Inducing TLS is time-consuming and has low success rates (118). The current understanding of TLS regulation has facilitated the development of immunotherapy based on TLS formation. However, the mechanisms underlying TLS formation during antitumor immune response should be further explored. Investigating TLS in HNSCC provides fresh insights into assessing prognosis and anticipating therapeutic reactions. Exploring the underlying processes of TLS development and operation could pave the way for innovative immunotherapy approaches, thereby boosting survival chances and therapeutic results for patients with HNSCC. Upcoming studies ought to delve deeper into the significance of TLS in various malignancies and determine methods to manipulate TLS to amplify anti-tumor immunity.

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Availability of data and materials

Not applicable.

Authors' contributions

SH and QW were involved in the conceptualization of the study and writing the original draft. DY and JZ collected information. JG was involved in reviewing and editing the manuscript. PH and YZ created the figures. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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