Therapeutic potential of tertiary lymphoid structures in breast cancer (Review)

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Abstract. Tertiary lymphoid structures (TLS) are organized immune aggregates within the tumor microenvironment that are associated with improved prognosis and immunotherapy response in breast cancer, yet their functional duality and therapeutic potential require further clarification. The present review comprehensively summarizes current knowledge on TLS in breast cancer, focusing on their molecular mechanisms of formation and maturation, multifaceted functions in anti-tumor immunity, established prognostic importance and emerging strategies for therapeutic targeting. The present study highlights that mature and functional TLS are key mediators of adaptive anti-tumor immunity, whereas immature TLS may have opposing effects. Their presence and characteristics hold strong prognostic value and can predict response to immune checkpoint inhibitors. Consequently, therapeutic strategies aimed at inducing or enhancing TLS represent a promising direction for breast cancer immunotherapy. However, further research is needed to elucidate the precise regulatory networks and translate TLS-focused strategies into clinical practice.

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Abbreviations: TLS, tertiary lymphoid structures; TME, tumor microenvironment; GC, germinal center; DC, dendritic cell; HEV, high endothelial venule; LTi, lymphoid tissue inducer; LTo, lymphoid tissue organizer; Tfh cell, follicular helper T cell; LTβR, lymphotoxin-β receptor; LTα, lymphotoxin-α; NK, natural killer; FDC, follicular dendritic cell; Treg, regulatory T cell; Trm, tissue-resident memory T cell; TIL, tumor-infiltrating lymphocyte; OS, overall survival; DFS, disease-free survival; TNBC, triple-negative breast cancer; ICI, immune checkpoint inhibitor; PNAd, peripheral node addressin; HEC, high endothelial cell; SHM, somatic hypermutation; CSR, class switch recombination; CAFs, cancer-associated fibroblasts

Key words: tertiary lymphoid structures, breast cancer, tumor microenvironment, tumor immunity, targeted therapy

Contents

1. Introduction

Breast cancer remains a notable global health challenge, being the most commonly diagnosed cancer and a leading cause of cancer-related mortality among women worldwide-in 2022, this burden translated to 2.3 million new cases (representing 25% of all cancers in women) and 670,000 deaths (representing 16% of all cancer-related deaths in women) (1). Despite advances in early detection and multimodal therapies, metastatic disease and therapeutic resistance continue to drive poor outcomes, underscoring the need for novel treatment strategies, particularly those that harness the immune system, such as immune checkpoint inhibitors or adoptive cell therapies, to enhance antitumor immunity.

The tumor microenvironment (TME) is a complex ecosystem that orchestrates tumor progression and response to therapy. Within this landscape, the immune cell compartment serves a dual role, capable of both suppressing and promoting tumor growth. Previous research has primarily focused on organized immune aggregates within the TME, known as tertiary lymphoid structures (TLS) (2,3). These ectopic lymphoid formations recapitulate key features of secondary lymphoid organs, comprising distinct T cell and B cell zones, germinal centers (GCs), dendritic cells (DCs) and specialized high endothelial venules (HEVs) that facilitate lymphocyte recruitment (4). Initially characterized

through research regarding chronic inflammatory and autoimmune diseases (5-7), TLS have become increasingly important in oncology across the past decade. Their identification within a number of solid tumors, including breast cancer, has advanced the general understanding of *in situ* anti-tumor immunity (8).

In breast cancer, the presence of TLS is frequently, though not exclusively, associated with a favorable prognosis and an enhanced response to immunotherapy, highlighting their potential as a biomarker and therapeutic target (9,10). However, their function is not monolithic; emerging evidence reveals a spectrum of maturity and activity, where only fully mature, functional TLS drive effective anti-tumor immunity, while immature aggregates may be inert or even pro-tumorigenic (11,12). This functional duality presents a critical clinical challenge: the mere presence of TLS cannot be uniformly interpreted as a positive sign, as their context-dependent and maturation-linked functions could lead to misleading prognostic conclusions if not properly distinguished (10). Conversely, it also unlocks a significant therapeutic opportunity: By deciphering the molecular switches that govern TLS maturation, strategies can be developed to selectively induce or stabilize mature, protective TLS, thereby converting an ambiguous histological feature into a precise, actionable target for cancer immunotherapy (11). Consequently, the present review provides a comprehensive and updated analysis of TLS in breast cancer, dissecting the molecular pathways governing their formation and maturation, elucidating their multifaceted functions within the TME, evaluating their prognostic importance and critically appraising strategies to therapeutically target TLS and further improve clinical outcomes.

2. Literature search

Comprehensive searches were conducted in PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://www.webofscience.com) and Scopus (https://www.scopus.com/). The core strategy combined terms related to ['Breast Cancer' (Title/Abstract) OR 'Breast Neoplasm' (Title/Abstract)] AND ['Tertiary Lymphoid Structures' (Title/Abstract) OR 'TLS' (Title/Abstract)]. The literature search was conducted in PubMed, Web of Science and Scopus from January 2015 to December 2025, covering all relevant articles published during this period. After removing duplicates, titles and abstracts were screened for relevance. Full texts of potentially eligible articles were then assessed against predefined inclusion and exclusion criteria. The inclusion criteria were: i) Original research articles or authoritative review papers; ii) studies investigating the biological mechanisms, prognostic significance or therapeutic role of TLS in breast cancer; iii) studies published in peer-reviewed journals; and iv) articles written in English. The exclusion criteria were: i) Case reports, conference abstracts, editorials, or commentaries without original data; ii) studies with insufficient data or unclear methodology; and iii) duplicate publications of the same data. Following this screening process, 67 studies met all eligibility criteria and were selected for critical analysis and inclusion in the present review.

3. Formation of TLS in breast cancer

Formation of TLS in breast cancer is a complex, multi-step process orchestrated by chronic inflammation and persistent antigen exposure. This cascade involves a coordinated network of immune and stromal cells, guided by precise molecular signals. The process is initiated when tumor-derived damage-associated molecular patterns and neoantigens trigger a pro-inflammatory microenvironment. This milieu, rich in cytokines such as TNF-α, IFN-γ, IL-1β and IL-6, serves as the initial signal that activates surrounding stromal and endothelial cells (13,14).

Following this initial inflammatory trigger, the organizational phase commences, relying not on a single cell type but on a collaborative cellular network. This network potentially re-activates an embryonic developmental pathway, involving lymphoid tissue inducer (LTi) and lymphoid tissue organizer (LTo) cells. Their interaction through the lymphotoxin-α (LTα)-1β2/lymphotoxin-β receptor (LTβR) signaling axis is key in the secretion of key lymphoid chemokines: C-X-C motif chemokine ligand 13 (CXCL13), which recruits B cells and a subset of T follicular helper (Tfh) cells; and C-C motif chemokine ligands 19 (CCL19) and 21 (CCL21), which recruit T cells and dendritic cells (DCs) (15,16). In the established TME, this foundation is augmented and dominated by adaptive immune mechanisms. The follicular helper T cell (Tfh)/B cell axis emerges as central in this process (17), whereby activated Tfh cells (themselves major producers of CXCL13) engage B cells through CD40L/CD40 and inducible T cell costimulator (ICOS) and its ligand ICOSL (ICOS ligand) co-stimulatory signals to drive the GC reactions key in TLS function (18,19). This adaptive cellular hub is supported by cancer-associated fibroblasts, which act as lymphoid stromal organizers by secreting CCL19, CCL21 and CXCL12 (20,21), as well as $CCL19^{+}$ DCs that recruit naïve T cells and help structure the T-cell zone (22,23).

Concurrent with cellular recruitment, the structural assembly of TLS is determined. This is primarily achieved through LTβR-driven neoformation of specialized HEVs, which are composed of high endothelial cells (HECs) that express the marker MECA79 (24). These endothelial cells are not passive conduits but active gateways that express peripheral node addressin (PNAd). PNAd comprises a set of glycosylated ligands recognized by CD62L (L-selectin) on circulating lymphocytes. This interaction mediates lymphocyte tethering and rolling along the vascular endothelium-the initial steps of lymphocyte recruitment into developing TLS, thereby contributing to their cellular accumulation and structural organization (25,26). Subsequent firm adhesion and transendothelial migration are further orchestrated by integrin activation (e.g., LFA-1/ICAM-1 interactions) and chemokine signals (e.g., CCL19, CCL21 or CXCL12) presented on the endothelial surface, which trigger conformational changes in integrins and direct lymphocyte migration into the developing TLS (13). This process is supported by vascular endothelial growth factor (VEGF) signaling, which acts directly on endothelial cells to promote their differentiation into the HEC phenotype, sustain their survival and enhance the expression of adhesion molecules (e.g., ICAM-1, VCAM-1, E-selectin and PNAd) and chemokines (e.g., CCL19, CCL21, CXCL12 and

CXCL13) that are key for lymphocyte recruitment (13,26). The spatial organization of the infiltrating cells is then meticulously directed by a precise chemokine gradient. First, CXCL13 attracts $CXCR5^+$ B cells and Tfh cells to form B cell follicles. Subsequently, CCL19 and CCL21 guide $CCR7^+$ T cells and DCs to the T-cell zone. Lastly, CXCL9, CXCL10 and CXCL11 induce $CXCR3^+$ effector T cells and natural killer (NK) cells, enhancing immune surveillance (22,27) (Fig. 1).

The culmination of this process is functional maturation, definitively marked by the establishment of an active GC. Within this specialized niche, Tfh-derived IL-21 and sustained co-stimulation drive B cells through clonal expansion, somatic hypermutation (SHM), class switch recombination (CSR) and final differentiation into antibody-secreting plasma cells and memory B cells, a process supported by a network of follicular DCs.

In summary, TLS formation in breast cancer is orchestrated through a dual-driven, collaborative network that bridges innate and adaptive immunity (Table I). This process unfolds sequentially through inflammatory initiation, chemokine-guided organization centered on Tfh cells and activated fibroblasts, as well as functional maturation driven by Tfh-B cell interactions (17,19). Notably, the trajectory and outcome of this process is markedly shaped by the molecular subtype and mutational load of the tumor, highlighting the context-dependent nature of antitumor immunity. Despite these advances, key knowledge gaps remain. First, the precise cellular origins and regulatory mechanisms of TLS-initiating cells (functionally analogous to LTi/LTo cells) in the established TME are still unclear. Second, the molecular drivers that determine whether TLS develop into functional or dysfunctional structures across different breast cancer subtypes remain poorly defined. Third, while key pathways such as LTβR and chemokine signaling have been delineated in preclinical models, their therapeutic relevance in patients requires further validation. Addressing these shortcomings is important in translating mechanistic insights into effective TLS-directed therapies.

4. Maturation of TLS in breast cancer

TLS are ectopic, organized immune aggregates that develop in non-lymphoid tissues under conditions of chronic inflammation, such as cancer, including non-small cell lung cancer, melanoma, colorectal cancer, breast cancer, renal cell carcinoma, hepatocellular carcinoma and bladder cancer (2,4,8). In breast cancer, TLS have been increasingly recognized as important hubs for initiating adaptive anti-tumor immunity (4,9,11). While resembling secondary lymphoid organs in cellular composition, TLS lack afferent lymphatics and a capsule. Notably, their presence and degree of maturation are associated with improved patient prognosis and enhanced responses to immunotherapy, underscoring their clinical relevance (28,29). The maturation of TLS is a dynamic continuum, transforming from a simple lymphocyte infiltrate into a highly organized, functional unit. This process is governed by the complex interplay of cellular interactions, molecular signals and structural evolution within TME. The TME itself can either promote or inhibit the formation and maturation of TLS, depending on the balance between pro-inflammatory and anti-inflammatory signals.

The maturation of TLS is driven by a core cellular collaborative network and precise molecular signals, with its ultimate morphological and functional hallmark being the formation of an active GC, which is associated with a favorable clinical prognosis (30,31). The central driver of this process is the Tfh cell/B cell axis, whereby B cells present antigens to Tfh cells, which in turn provide essential co-stimulatory signals such as CD40L and the key cytokine IL-21, establishing a positive feedback loop that drives B cell clonal expansion, SHM and affinity maturation (17,18). The establishment and spatial organization of this collaborative network relies on a well-defined chemokine gradient. This involves CXCL13 (mainly derived from Tfh cells and stromal cells) recruiting $CXCR5^+$ B cells and Tfh cells, laying the foundation for B cell follicles/GCs. Conversely, CCL19/CCL21 (derived from DCs and fibroblasts) guide $CCR7^+$ T cells and DCs to the T cell zone, thereby completing this functional zoning and structural assembly of the entire TLS (22,25).

Based on histopathological features, the maturation of TLS progresses through a continuous spectrum from a simple inflammatory infiltrate to a fully functional ectopic lymphoid organ, which can be categorized into three sequential stages (25). The process begins with the early/immature TLS stage, characterized by disorganized, diffuse aggregates of lymphocytes lacking distinct zoning and a GC, accompanied by sparse or underdeveloped HEVs. As maturation advances to the intermediate/developing TLS stage, initial spatial organization emerges with the formation of separate yet rudimentary T cell and B cell zones, the beginning of follicular DC (FDC) network formation and more prominent HEVs that facilitate enhanced lymphocyte recruitment. The culmination of this process is the late/mature TLS stage, marked by the presence of a fully formed, active GC within the B cell zone, exhibiting clear dark and light zones, a surrounding mantle zone and a distinct T-cell area. This final, mature stage has been consistently associated with the most robust anti-tumor immune responses and the most favorable clinical outcomes. In breast cancer, mature TLS support germinal center-driven B-cell differentiation, antibody production and enhanced cytotoxic T cell activity (17-19). Clinically, their presence correlates with prolonged overall survival (OS) and disease-free survival (DFS). Furthermore, it predicts improved response to immune checkpoint inhibitors (26-29). The maturation stage of TLS can be delineated using immunohistochemistry or multiplex fluorescence, with key markers including Ki-67 to identify proliferating GC B cells (3), activation-induced cytidine deaminase as a specific marker for ongoing SHM and class-switch recombination within the GC (4), B cell lymphoma 6 (BCL6) for GC activity, CD20 for B cells, CD23/CD21 for FDC networks, as well as CD8 for cytotoxic T cells, CD138 for plasma cells and DC-lysosomal associated membrane protein for mature DCs (10) (Fig. 2).

Collectively, TLS maturation constitutes a functional continuum in breast cancer. Progression to a germinal center-positive and mature TLS, signifies an immunocompetent TME that can sustain potent adaptive immunity. Therefore, assessing the maturation stage offers greater prognostic and predictive value compared with simply documenting TLS presence. Consequently, strategies to induce or stabilize

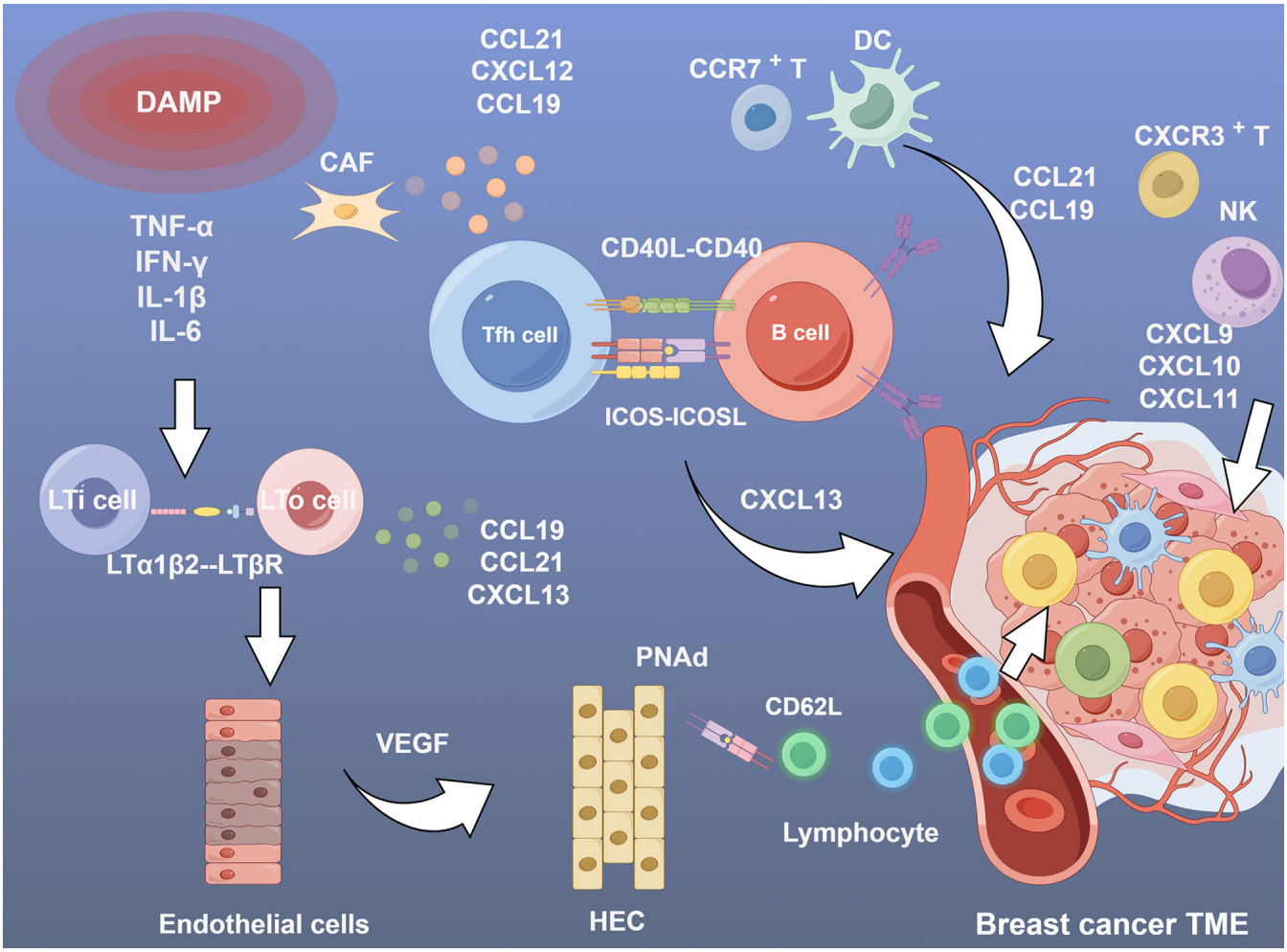

Figure 1. Molecular cascade driving TLS formation in breast cancer. Schematic illustrating that the formation of TLS is a multi-step process initiated by tumor-derived DAMPs and neoantigens, which promote a pro-inflammatory microenvironment rich in cytokines such as TNF-α, IFN-γ, IL-1β and IL-6. This inflammatory milieu activates stromal cells, including fibroblasts and endothelial cells. Activated fibroblasts act as lymphoid stromal organizers by secreting CCL19, CCL21 and CXCL12. The organizational phase involves a cellular network where LTi cells interact with LTo cells (including fibroblasts) via the LTα1β2-LTβR axis, leading to the secretion of homeostatic chemokines (CXCL13, CCL19 and CCL21). This is reinforced by adaptive immunity, particularly the Tfh cell/B cell axis, where Tfh cells (producing CXCL13) provide CD40L and ICOS co-stimulation to B cells to drive germinal center reactions. Concurrently, LTβR signaling drives the differentiation of endothelial cells into specialized HECs. These HECs express PNAd, which interacts with CD62L on lymphocytes to facilitate homing, a process supported by VEGF. The spatial organization of infiltrating immune cells is directed by chemokine gradients: CXCL13 recruits $CXCR5^+$ B cells and Tfh cells to follicles; CCL19 and CCL21 recruit $CCR7^+$ T cells and DCs to the T cell zone; and CXCL9, CXCL10 and CXCL11 recruit $CXCR3^+$ T cells and NK cells. TLS, tertiary lymphoid structures; DAMP, damage-associated molecular pattern; LTi, lymphoid tissue inducer; LTo, lymphoid tissue organizer; HEC, high endothelial cells; TME, tumor microenvironment; LTα, lymphotoxin-α; LTβR, lymphotoxin-β receptor; Tfh, follicular helper T cell; PNAd, peripheral node addressin; DC, dendritic cell; NK, natural killer; CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; ICOS, inducible T cell costimulatory; ICOSL, ICOS ligand; CAF, cancer-associated fibroblast.

mature TLS represent a promising avenue for improving immunotherapy outcomes.

However, translating TLS maturation into a reliable clinical biomarker and therapeutic target faces a number of unresolved challenges. First, existing histopathological staging systems (early, intermediate and mature) lack standardized, quantitative criteria, introducing assessment variability. Second, while GC presence defines maturity, the prognostic impact of GC features, such as size, proliferative activity (including Ki-67 and BCL6) as well as plasma cell output, requires standardization. Third, current static histological snapshots cannot capture TLS dynamics over time or in response to treatment, necessitating the development of non-invasive imaging or circulating biomarkers for longitudinal monitoring. Addressing these gaps is key in fully leveraging TLS maturity in precision immuno-oncology.

5. Function of TLS in breast cancer

Functional roles of TLS in breast cancer are not monolithic, but exist along a dynamic spectrum, determined by their stage of maturation (25,30). This functional duality means TLS can act either as a potent ally or a paradoxical antagonist in the anti-tumor immune response, with their structural maturity serving as the decisive switch (32,33). Immature TLS, characterized by disorganized immune cell aggregates devoid of GCs, often represent a dysfunctional and potentially harmful niche. These structures are frequently enriched in immunosuppressive cell types such as regulatory T cells (Tregs) and exhibit high expression of inhibitory molecules [including programmed death ligand 1 (PD-L1), IL-10 and TGF-β]. This milieu inhibits effector T cell function, fosters

Table I. Key molecular regulators of TLS formation and maturation in breast cancer.

Category	Key molecules	Primary cellular source	Function and mechanism	Supporting evidence in breast cancer	(Refs.)
Initiating and organizing cytokines	Lymphotoxin α/β	LTi cells and activated lymphocytes	Binds LTβR to induce stromal cell differentiation and *de novo* HEV formation	Positively associated with TLS density; high expression predicts favorable prognosis	(25)
	CXCL13	LTi cells, Tfh cells and CAFs	Recruits $CXCR5^+$ B cells and Tfh cells to form the B-cell follicle	A hallmark chemokine of TLS; predicts response to immunotherapy	(25)
	CCL19/CCL21	Stromal cells and DCs	Recruits $CCR7^+$ T cells and DCs to organize the T cell zone	Highly expressed in TLS-positive tumors	(22)
Vascular remodeling factor	VEGF	Tumor cells, CAFs and macrophages	Promotes endothelial cell survival, proliferation and differentiation into HEVs	Associated with HEV density; anti-VEGF therapy may modulate TLS	(27)
Germinal center functional molecules	AID	GC B cells	Mediates SHM and CSR for antibody affinity maturation	Present in mature TLS; linked to B-cell anti-tumor activity	(5)
	BCL6	GC B cells and Tfh cells	Master transcriptional regulator required for establishing and maintaining the GC reaction	Serves as a marker for GC formation within TLS in breast cancer	(10,19)

CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; AID, activation-induced cytidine deaminase; GC, germinal center; LTβR, lymphotoxin-β receptor; TLS, tertiary lymphoid structures; DC, dendritic cell; Tfh, follicular helper T cell; CAF, cancer-associated fibroblast; HEV, high endothelial venule; LTi, lymphoid tissue inducer; SHM, somatic hypermutation; CSR, class switch recombination; VEGF, vascular endothelial growth factor.

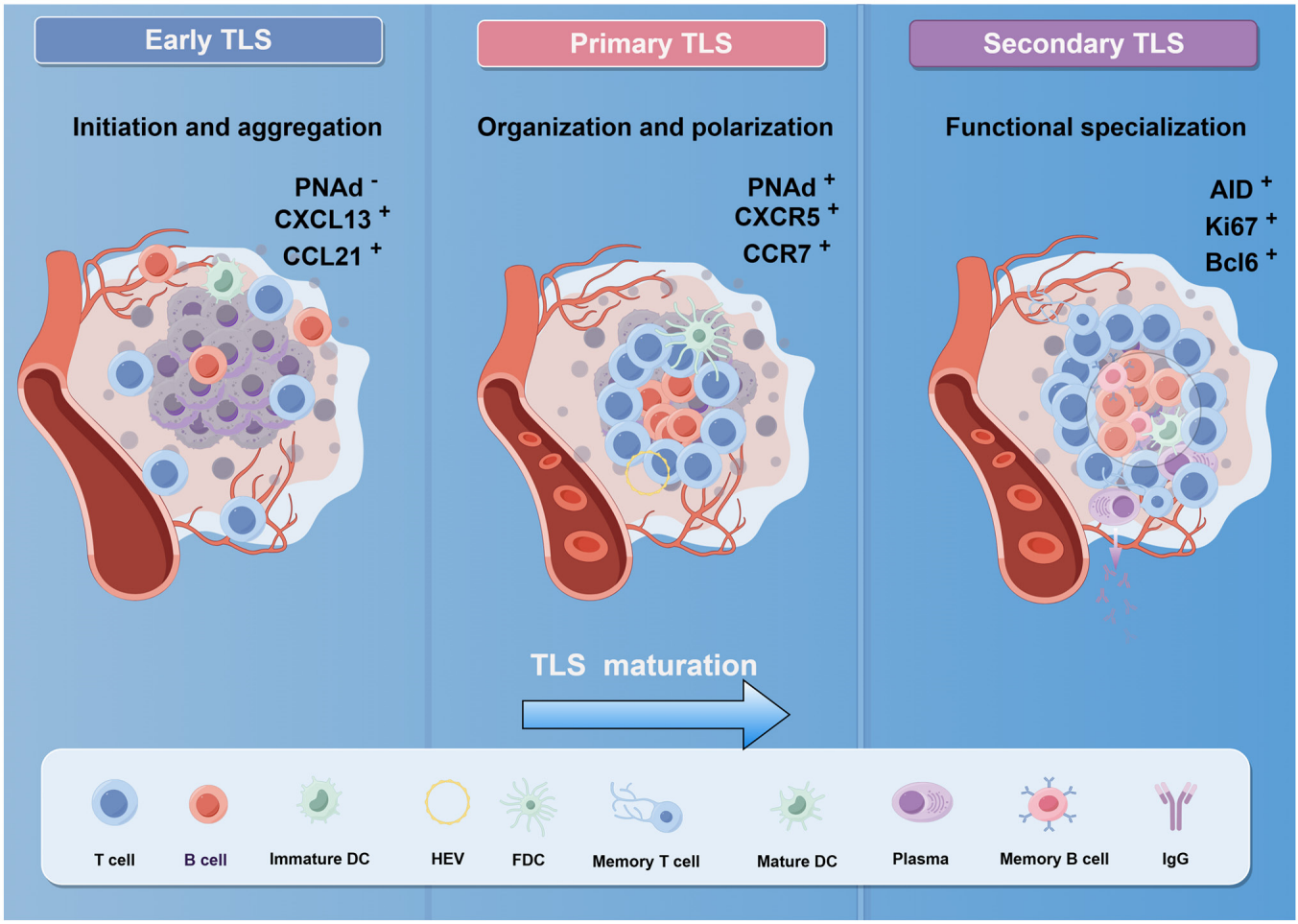

Figure 2. Progressive maturation of TLS in breast cancer. Figure depicting the three-stage evolution of TLS from initial aggregation to a fully functional, organized ectopic immune niche. Early stage (aggregate): Diffuse infiltration of immune cells, including $CD3^+$ T cells, $CD20^+$ B cells and $CD11c^+$ DCs, is driven by high expression of the chemokines CXCL13 and CCL19/21. This stage is characterized by the absence of spatial organization and the presence of only conventional $CD31^+$ blood vessels lacking the PNAd, indicating immature lymphocyte homing capacity. Primary stage (structured): Sustained LTβR signaling drives the formation of specialized $PNAd^+$ $MECA79^+$ high HEVs, establishing an efficient portal for lymphocyte entry. Initial compartmentalization emerges, with $CXCR5^+$ B and T follicular helper cells beginning to coalesce into a B cell follicle, while $CCR7^+$ T cells and dendritic cells organize into an adjacent T cell zone guided by a CCL21 gradient. Secondary stage (GC-positive): TLS achieves full architectural and functional maturity. A distinct GC forms within the B cell follicle, evidenced by expression of AID, the transcriptional regulator BCL6 and the proliferation marker Ki-67. This results in the output of IgG^+ antibody-secreting plasma cells and the generation of tissue-resident memory T cells. TLS, tertiary lymphoid structures; PNAd, peripheral node addressin; DC, dendritic cell; CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; LTβR, lymphotoxin-β receptor; HEV, high endothelial venule; CCR, C-C motif chemokine receptor; AID, activation-induced cytidine deaminase; GC, germinal center. FDCs, follicular dendritic cells.

tumor immune evasion and may promote progression, possibly accompanied by abnormal angiogenesis driven by elevated VEGF expression (34). By contrast, mature TLS, defined by segregated B cell and T cell zones housing an active GC, function as sophisticated command centers for adaptive immunity (25). Their potent anti-tumor mechanisms are multi-pronged, encompassing both cellular and humoral arms. Within the T cell zone, efficient antigen presentation drives the clonal expansion of tumor-specific cytotoxic T cells ($CD8^+$) and memory T cells (14,22). Concurrently, in the GC, B cells undergo affinity maturation and CSR to produce high-affinity antibodies. These antibodies can orchestrate tumor cell elimination through several key effector mechanisms including antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis and complement-dependent cytotoxicity (35). Furthermore, the cytokine and chemokine milieu (such as IFN-γ and CXCL13) secreted by mature TLS, recruits and activates additional effector cells including NK cells and macrophages, amplifying the local immune attack and potentially seeding systemic immune surveillance through circulating effector T cells (13,17) (Fig. 3).

Clinically, this functional divergence translates into important prognostic implications. The presence of mature TLS is associated with improved OS and DFS, particularly in aggressive subtypes such as triple-negative breast cancer (TNBC) and HER2-positive breast cancer (28,36,37). In addition, TLS maturity serves as a biomarker predictive of enhanced response to immunotherapy, indicating a pre-existing, immunocompetent TME (29,31,38), a concept which has brought attention to TLS, facilitating therapeutic research. Preclinical strategies have aimed to induce or stabilize mature TLS through local delivery of lymphoid chemokines/cytokines including lymphotoxin (39), use of engineered dendritic cell vaccines (40) or rational combination with immune checkpoint inhibitors (41,42). These

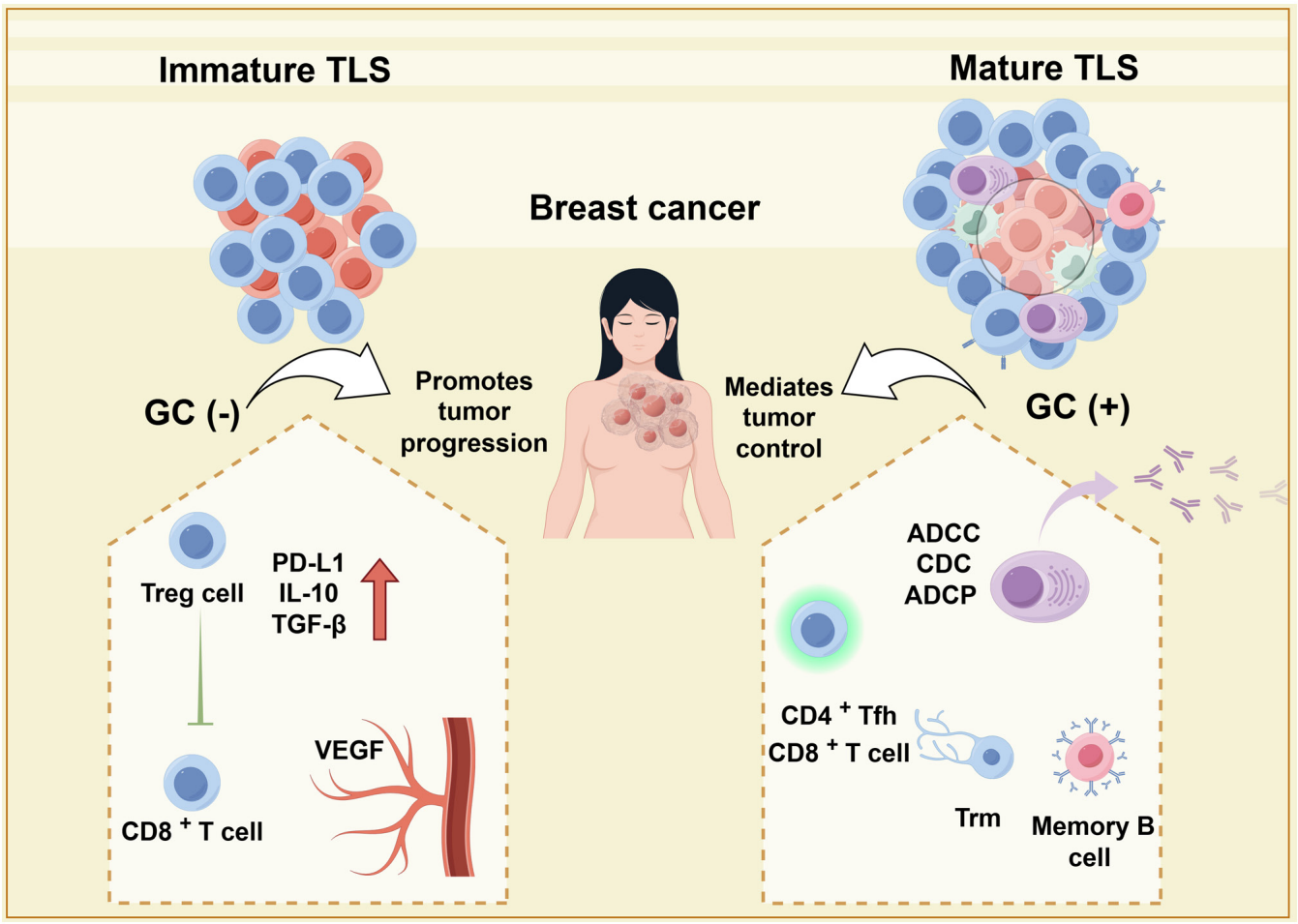

Figure 3. Dual functional roles of TLS in breast cancer progression and control. TLS exert opposing effects on the tumor microenvironment depending on their maturation state. Immature TLS are structurally disorganized and devoid of germinal centers. Immature TLS are often enriched in immunosuppressive cell types such as Tregs and exhibit high expression of inhibitory molecules (including PD-L1, IL-10 and TGF-β). This milieu inhibits effector T cell function and fosters an environment that promotes tumor immune evasion and progression or accompanied by abnormal angiogenesis promoted by high expression of VEGF. Mature TLS are highly organized, containing a germinal center that supports antibody affinity maturation and the generation of plasma cells secreting high-affinity IgG. Concurrently, mature TLS facilitate the activation of cytotoxic $CD8^+$ T cells and the formation of Trm. Through these coordinated adaptive immune responses, mature TLS effectively mediate tumor cell killing and immune control (for example, ADCC, CDC and ADCP). The balance between these divergent functional states markedly influences clinical outcomes. TLS, tertiary lymphoid structures; Tregs, regulatory T cells; PD-L1, programmed death ligand 1; Trm, tissue-resident memory T cells; GC, germinal center; Tfh, follicular helper T cell; VEGF, vascular endothelial growth factor; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity.

methods have demonstrated promising efficacy in inhibiting tumor growth. Therefore, the functional evaluation of TLS must evolve beyond a binary assessment of their presence to a precise grading of their maturation stage. Therapeutically, promoting the transformation of immature, dysfunctional TLS into their mature, functional counterparts, represents an important strategy in reprogramming the tumor immune landscape and unlocking effective anti-tumor immunity, which may hold marked promise for improving outcomes in patients with breast cancer (19,43).

Briefly, in breast cancer, TLS function with a dual role which is critically determined by their maturation stage (4,30). Immature TLS, characterized by disorganized architecture, can foster local immunosuppression. By contrast, mature TLS serve as coordinated hubs for adaptive immunity, integrating cellular and humoral anti-tumor responses. Clinically, the presence of mature TLS is a notable biomarker associated with improved prognosis and enhanced sensitivity to immunotherapy. Therefore, future efforts should aim to prioritize accurately evaluating TLS maturity and developing strategies to induce functional maturation, thereby reprogramming the immune TME.

However, translating this functional paradigm into robust clinical application faces a number of key challenges. First, the molecular cues that drive an immature TLS toward a functional immune hub vs. a dysfunctional, immunosuppressive niche are poorly understood. Second, the majority of evidence associating TLS maturity with outcomes derives from retrospective studies; prospective validation in large, uniformly treated cohorts is needed to establish its independent predictive value. Finally, while inducing TLS maturation is a compelling therapeutic goal, current strategies are primarily preclinical and their ability to generate stable, functional and safe TLS in patients remains unproven. Addressing these gaps is therefore necessary in advancing TLS from a descriptive biomarker to a tractable component of precision cancer immunotherapy.

6. Prognostic importance of TLS in breast cancer

Any prognostic value of TLS with regard to breast cancer is multifaceted and extends beyond a simple assessment of their presence or absence. Their predictive power is derived from a profile encompassing structural maturity, spatial distribution, cellular composition and density. Collectively, these features define the functional capacity of TLS within TME and are therefore associated with clinical outcomes.

A central prognostic determinant is TLS structural maturity. Fully mature TLS, characterized by segregated B cell and T cell zones with active GCs, function as organized hubs for adaptive immunity, consistently associating with favorable clinical outcomes and improved responses to immunotherapy (25,30,31). By contrast, immature TLS, namely disorganized lymphoid aggregates lacking GCs, may be functionally inert or even foster a pro-tumorigenic, immunosuppressive niche (32,34). Further refinement comes from their cellular composition whereby a predominance of cytotoxic $CD8^+$ T cells may be associated with protective immunity, while a high density of Tregs may suppress it (19).

Spatial localization and density add further prognostic nuance. TLS at the invasive tumor margin (peritumoral) are often more associated with positive outcomes compared with intratumoral TLS, likely due to enhanced access to circulatory immune cells (32). In addition, TLS density frequently serves as a stronger prognostic indicator compared with simply the presence of TLS, with higher density having been associated with improved survival (28,36). However, this is context-dependent, as abundant but immature peritumoral TLS may be associated with aggressive features and worse prognosis, underscoring the necessity of evaluating maturity alongside location (24,32).

TLS exist within a broader immune context. While often associated with high overall tumor-infiltrating lymphocyte (TIL) density, beneficial subsets including $CD8^+$ T cells and Th1 cells (14), TLS presence itself has been identified as an independent favorable prognostic factor in HER2-positive breast cancer, irrespective of total TIL levels (37,44). Furthermore, a key structural component, mature HEVs within TLS, are themselves, a marker of favorable prognosis due to their key role in lymphocyte recruitment (25,26).

Clinical meta-analyses have consolidated the positive prognostic role of TLS. An analysis of nine studies involving 2,281 patients determined that TLS presence was markedly associated with improved OS and DFS (36). This prognostic benefit extends across major molecular subtypes, including TNBC, HER2-positive and hormone receptor-positive breast cancer (36,37,45).

In summary, TLS are sophisticated prognostic biomarkers in breast cancer. Their accurate interpretation requires a layered assessment that integrates structural maturity, spatial distribution, density and cellular composition. The most favorable prognostic profile is associated with mature, dense, peritumoral TLS enriched with cytotoxic T cells, reflecting a highly active and coordinated anti-immune TME. Translating this knowledge into clinical utility necessitates the development and adoption of a standardized TLS grading system for patient stratification and therapeutic optimization.

Despite this, a number of methodological and conceptual limitations must be addressed to clarify the prognostic value of TLS. As aforementioned, one primary challenge is the absence of a universally accepted, integrative grading system that reliably scores key features, hindering reproducible assessment and cross-study comparisons. Furthermore, while meta-analyses have supported a positive prognostic trend, the majority of evidence is retrospective; prospective validation in large, contemporary cohorts managed with current standards of care (including immunotherapy) is needed. The prognostic weight of specific TLS features-including structural maturity (e.g., presence of germinal centers, segregated B/T cell zones), spatial distribution (peritumoral vs. intratumoral), cellular composition (e.g., ratios of $CD8^+$ T cells, Tregs, B cells) and density-may also vary notably across molecular subtypes and treatment contexts, a heterogeneity that remains insufficiently characterized. Resolving these limitations is key in refining TLS into a precise and actionable tool for clinical decision-making. Lastly, the growing recognition of the prognostic and predictive role of TLS has underscored the need for reliable detection methods (12,32,33,36,39,45). A comparative overview of key techniques for TLS identification in breast cancer is provided in Table II, highlighting their respective advantages and limitations.

7. Potential of TLS as a therapeutic target for breast cancer

Investigating the immunogenic potential of TLS represents a promising avenue in breast cancer therapy. The core rationale is to reprogram the immunosuppressive TME by inducing, maturing or functionally modulating these ectopic lymphoid aggregates, thereby fostering a coordinated adaptive immune response that synergizes with existing treatments (41,42,46). A growing body of evidence has indicated that immune checkpoint inhibitors actively shape the immune TME by promoting the neogenesis and maturation of TLS (38,47,48). The primary mechanism is considered to be the reversal of T cell exhaustion and the alleviation of immunosuppression within pre-existing lymphoid aggregates, which facilitates the coordinated action of key TLS-resident cells such as Tfh cells and germinal center B cells (17,44). In breast cancer, patients with objective response (complete or partial response) to programmed cell death protein 1 (PD-1)/PD-L1 blockade often exhibit increased TLS density and maturity, suggesting TLS formation is a dynamic process enhanced by therapy (29). This role of immune checkpoint inhibitors in fostering TLS is further evidenced by studies in other solid tumors, such as melanoma and non-small cell lung cancer, whereby treatment with immune checkpoint inhibitors has been directly associated with *de novo* TLS induction and increased lymphoid organization (35,49,50). Consequently, TLS are not just static biomarkers but active, inducible mediators of the anti-tumor immune response. The efficacy of immune checkpoint inhibitors may depend, in part, on a functional TLS compartment, specifically, the presence of mature B-cell follicles with active germinal centers, positioning these structures as key determinants of treatment success (51,52). This understanding informs future therapeutic strategies, such as combining PD-1/PD-L1 blockade with inhibitors of other immune checkpoints enriched in

Table II. Comparative analysis of the advantages and disadvantages of methods for detecting TLS in breast cancer.

Method	Advantages	Disadvantages
Flow cytometry	High-throughput; quantitative; multi-parameter analysis	Requires fresh/frozen cells; antibody-dependent; no morphological information
Immunohistochemistry	Morphological visualization; FFPE-compatible; good specificity and sensitivity	Manual and time-consuming; inter-observer variability; antibody optimization often required
H&E	Routine, widely available; morphological context; low cost	Low sensitivity/specificity for TLS; subjective interpretation; no immune cell phenotyping
Multiplex immunofluorescence	Simultaneous multi-marker visualization; high sensitivity and specificity	Technically complex; expensive; requires specialized equipment and expertise
Gene TLS signature for quantification	Predicts prognosis and immunotherapy response; associates with molecular subtype, TME, TMB; associated with improved OS	Requires transcriptomic data; limited applicability in some settings due to technical constraints

TLS, tertiary lymphoid structures; FFPE, formalin-fixed paraffin-embedded; TME, tumor microenvironment; TMB, tumor mutational burden; OS, overall survival.

TLS (including lymphocyte activating 3 or T cell immunoglobulin and mucin-domain containing 3), to achieve more comprehensive immune reactivation within these pivotal ecosystem hubs (53,54).

Engineering the chemokine milieu represents a direct strategy for inducing TLS formation *de novo*. Preclinical studies have demonstrated that the localized delivery of key lymphoid chemokines, such as CCL19, CCL21 (ligands for CCR7) and CXCL13 (a ligand for CXCR5), can orchestrate the coordinated recruitment of T cells, DCs and B cells, thereby initiating the assembly of structured TLS (22,27,40). For example, local CCL21 expression not only promotes TLS formation alongside HEVs but is also an important step in establishing a supportive lymphoid niche (26). Similarly, modulation of the CXCL12/CXCR4 axis has been shown to influence the organization and spatial distribution of TLS within TME (42). Specifically, CXCL12, often secreted by cancer-associated fibroblasts (CAFs) and endothelial cells within the TME, binds to CXCR4 expressed on lymphocytes, thereby directing their migration and positioning. This chemotactic gradient facilitates the clustering of $CXCR4^+$ T cells and B cells into discrete perivascular niches, promoting their aggregation into nascent TLS (42). Furthermore, the CXCL12/CXCR4 axis contributes to the maintenance and spatial architecture of mature TLS by anchoring lymphocyte subsets within specific compartments and supporting the formation of HEVs, which are critical for sustained lymphocyte recruitment (20,24). Consequently, targeted modulation of this axis can alter TLS density, cellular composition and intratumoral localization, thereby shaping their functional status.

Cytokine and cellular therapies are being developed to specifically alter TLS function. Key cytokines intrinsic to TLS biology, such as IL-21 (produced by Tfh cells) and LTα, are being explored as therapeutic agents or targets to enhance TLS activity (17,39). Although systemic administration of cytokines such as IL-2 or IL-12 faces challenges due to potential toxicity, as their use aims to broadly stimulate T and NK cells that can subsequently seed or amplify responses within TLS (40). More sophisticated approaches, including adoptive T-cell transfer and dendritic cell vaccines, have been designed to deliver tumor-specific effector cells or antigen-presenting cells capable of trafficking to and selectively localizing within TLS, where they can amplify the local immune response (18,40,55). Furthermore, tumor antigen-specific vaccines aim to provide a sustained antigen source to fuel the GC reaction within TLS (56).

In addition, one strategic approach to TLS-directed therapy involves its combination with conventional treatments, leveraging their ability to remodel the TME in favor of lymphoid neogenesis (57,58). Chemotherapy can induce immunogenic cell death, thereby releasing tumor antigens that may serve as a sustained source for presentation within TLS. Radiotherapy can promote a localized, pro-inflammatory niche that enhances lymphocyte recruitment and promotes the stabilization of nascent TLS structures, defined as early lymphoid aggregates with nascent HEV formation and initial stromal remodeling, which represent the precursor stage to fully mature TLS (38,59). Furthermore, anti-angiogenic therapy can normalize the disordered tumor vasculature and facilitate the transformation of blood vessels into functional HEVs (24,41), which are a key structural prerequisite for lymphocyte entry and TLS organization. Preclinical evidence supports this integrative strategy; for example, the combination of anti-PD-L1 with anti-angiogenic therapy has been shown to synergistically drive HEV transformation and the consequent development of functional TLS (41) (Fig. 4).

In conclusion, targeting TLS signifies a shift from broad immune stimulation to the precise architectural engineering of the immune TME. Current strategies are multifaceted, including checkpoint modulation, chemokine/cytokine delivery, cellular therapies and synergistic combinations with conventional treatments. Future progress relies on identifying predictive biomarkers, optimizing localized delivery and designing clinical trials that incorporate advanced TLS phenotyping as correlative endpoints. The overarching goal is to foster '*in situ* immune factories' that remodel immunosuppressive landscapes into organized, functional hubs for sustained anti-tumor activity (Table III).

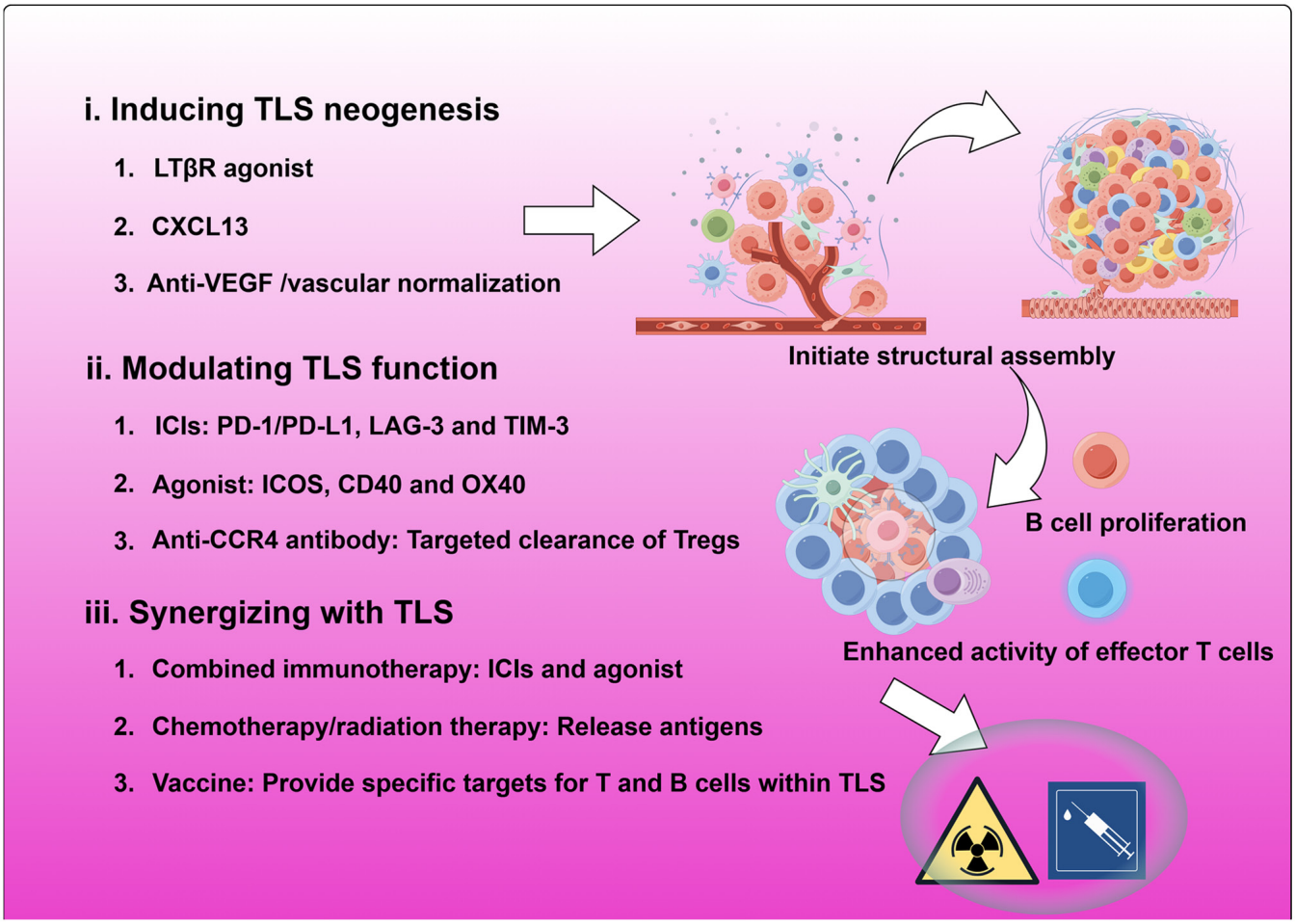

Figure 4. Therapeutic strategies targeting tertiary lymphoid structures in breast cancer. Schematic outlining a multi-pronged translational approach to harness TLS for cancer therapy. Strategies are categorized based on their primary objective; i) Inducing TLS neogenesis: Aims to initiate *de novo* TLS formation in non-inflamed tumors using agonists of key organizing pathways (such as LTβR and CXCL13) or vascular normalizing agents (anti-VEGF); ii) modulating TLS function: Seeks to convert existing TLS into potent anti-tumor hubs by blocking inhibitory checkpoints (including anti-PD-1), providing co-stimulatory signals (for example, anti-CD40) or depleting immunosuppressive cells (such as Tregs); and iii) synergizing with TLS: Leverages the presence of a functional TLS to enhance the efficacy of conventional therapies, such as chemo-/radiotherapy (which release tumor antigens) or cancer vaccines (which provide specific targets). Collectively, these strategies converge on the goal of amplifying adaptive anti-tumor immunity, including high-affinity antibody production and cytotoxic T cell activity, to ultimately improve clinical outcomes. TLS, tertiary lymphoid structures; Tregs, regulatory T cells; PD-L1, programmed death ligand 1; LTβR, lymphotoxin-β receptor; CXCL, C-X-C motif chemokine ligand; CCR, C-C motif chemokine receptor; ICOS, inducible T cell costimulatory; TIM-3, T cell immunoglobulin and mucin-domain containing 3; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; ICIs, immune checkpoint inhibitors; VEGF, vascular endothelial growth factor.

However, translating these preclinical concepts into safe and effective clinical therapies faces marked challenges. Safety concerns, particularly the risk of autoimmunity or uncontrolled inflammation from induced lymphoid neogenesis, require careful evaluation. Furthermore, therapeutic efficacy may be highly context-dependent, influenced by tumor subtype, baseline immunity and prior treatments; predictive biomarkers to identify ideal candidates are currently lacking. Finally, robust evidence from large, randomized clinical trials with TLS induction as a defined endpoint is needed to validate this therapeutic approach. Addressing these issues is important in realizing the full potential of TLS-directed therapy in breast cancer.

8. Discussion and future perspectives

Within the present review, a systematic summary of the formation, maturation, function, prognostic relevance and therapeutic potential of TLS in breast cancer is provided. By integrating evidence (including key studies ≤2024-2025), the present review aimed to advance the perspective of TLS from a static histological biomarker to a dynamic and therapeutically targetable immune niche within the TME. Convergent evidence has determined that TLS are organized functional hubs for adaptive anti-tumor immunity. Their maturation stage represents a key functional divide: Mature TLS with active GCs associate with favorable prognosis and response to immunotherapy (25,29-31), whereas immature TLS may be functionally impaired or even foster immunosuppression (32,34). This functional duality underscores the need to move beyond a binary assessment of TLS presence, toward a more evaluative framework that includes architecture, spatial location, density and cellular composition for precise prognostic stratification (28,36,37).

Despite this, notable challenges and open questions remain. First, the regulatory network controlling TLS

Table III. Treatment strategies targeting TLS in cancer therapy.

First author, year	Treatment strategy	Key agents/targets	Proposed mechanism of action in TLS	(Refs.)
Wu *et al*, 2023	Chemokine/cytokine-based therapy	CCL19, CCL21 and CXCL12	Promotes immune cell migration, positioning and communication within the TLS microenvironment (the CCL21/CCR7 axis)	(22)
Shiao *et al*, 2024	Immune checkpoint inhibitors	Nivolumab, pembrolizumab and tezolizumab	Blocks PD-1/PD-L1 or CTLA-4 inhibitory signals, thereby activating immune cells within TLS	(38)
Bertucci *et al*, 2021	Combination therapy	Anti-CTLA-4 and anti-PD-1/PD-L1, as well as rituximab	Induces TLS formation and potentiates anti-tumor immunity through synergistic mechanisms	(52)
Acar *et al*, 2022	Other immune checkpoint modulators	LAG-3, TIM-3 and TIGIT	Targets additional inhibitory or stimulatory pathways to relieve immune suppression and enhance T cell function within TLS	(54)

CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; TLS, tertiary lymphoid structures; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; CCR, C motif chemokine receptor; LAG-3, lymphocyte activation gene 3; TIM-3, T cell immunoglobulin and mucin-domain containing 3; TIGIT, T cell immunoreceptor with Ig and ITIM domains; CTLA, cytotoxic T lymphocyte associated protein.

formation is not fully elucidated. While key pathways such as LTβR signaling, CXCL13 and CCL19/CCL21 have been identified (22,25,60), it is unclear whether their activation and maintenance differ across breast cancer subtypes or how tumor cells actively modulate TLS neogenesis. Second, the origins of TLS functional heterogeneity require clarification. Why TLS are occasionally associated with a worse prognosis in certain contexts, such as some HER2-negative subtypes (24,32) may relate to the accumulation of immunosuppressive cells (such as Tregs, M2 macrophages) within TLS or aberrant crosstalk with specific stromal components (for example, immature vasculature). Further developments will likely come from single-cell and spatial multi-omics approaches (61,62). Furthermore, the absence of a standardized evaluation system represents a translational bottleneck. Inconsistent criteria for grading TLS maturity and quantifying TLS density hinder direct comparisons across studies (63,64).

Therapeutic translation, though promising, is complex. Strategies to induce TLS, via immune checkpoint inhibitors, chemokines, cytokines or vaccines, exhibit potential in enhancing anti-tumor immunity in preclinical models (40-42,65). However, translating these into safe and effective clinical applications poses considerable challenges. Systemic delivery of chemokines or cytokines, for example, carries risks of marked toxicity. It also remains uncertain whether induced TLS will consistently mature into functional, immunostimulatory hubs rather than dysfunctional or immunosuppressive aggregates. In addition, the functional and phenotypic equivalence between therapy-induced TLS and their counterparts is yet to be established (58,59). Future clinical trials should therefore aim to incorporate dynamic monitoring of TLS phenotype (maturity and composition) to clarify their role as pharmacodynamic biomarkers.

A number of key priorities have emerged for future research and clinical translation. Mechanistic investigation should leverage advanced tools such as spatial transcriptomics, single-cell sequencing and organoid co-culture systems to fully decipher the cellular crosstalk and spatiotemporal dynamics within TLS. Standardization and automation are urgently needed, requiring the establishment of international consensus guidelines for TLS histopathological assessment and the development of artificial intelligence-driven digital pathology tools to enable objective, reproducible quantification (46,66). In the realm of innovative therapeutics, there is a clear need to explore localized delivery systems (such as nanoparticles or oncolytic viruses) to target TLS-inducing factors (including LTα and CXCL13) specifically to the tumor site, with the aim of generating functional TLS while minimizing systemic exposure (40,42). Finally, the optimization of combination strategies must systematically determine the most effective sequencing and integration of TLS-inducing approaches with conventional therapies (chemotherapy, radiotherapy and targeted therapy) and other immunotherapies (such as bispecific antibodies and chimeric antigen receptor T cell therapy) to achieve synergistic anti-tumor effects (31,41,57).

Overall, TLS research is evolving from descriptive associations to mechanistic insight and therapeutic targeting. Elevating TLS from a passive prognostic indicator to an active therapeutic asset marks a framework shift in cancer immunotherapy. Addressing the aforementioned challenges through interdisciplinary collaboration will be key in fully realizing the potential of TLS, allowing for more effective and personalized treatments for patients with breast cancer, particularly those who derive limited benefit from current immunotherapies.

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Authors' contributions

LZ wrote the initial draft of the manuscript. ML interpreted literature data to design the key mechanistic diagrams, critically revised the main text in response to reviewers' comments, and takes accountability for the accuracy of the figures and the revised text. FL and FG contributed to the study design and final manuscript preparation. Data authentication is not applicable. All authors 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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