Chen *et al. Journal of Translational Medicine* (2025) 23:528
https://doi.org/10.1186/s12967-025-06510-6

Journal of Translational Medicine

REVIEW Open Access

Tertiary lymphoid structures in gliomas: impact on tumour immunity and progression

Jiatong Chen[5†], Yuechao Yang[1,3†], Shuang Luan[4†], Wenhao Xu[2,3*] and Yang Gao[1,3*]

Abstract
Tertiary lymphoid structures (TLSs) are ectopic lymphoid formations that develop in chronically inflamed tissues, including various solid tumours. In the context of gliomas, the presence of TLSs has recently attracted considerable attention because of their potential implications in tumour immunology and therapy. The tumour immune microenvironment (TIME) plays a crucial role in cancer progression, and tumour-infiltrating immune cells (TILs) are key players in this environment. These immune cell aggregates, known as TLSs, display distinct characteristics across different solid tumours. However, central nervous system (CNS) tumours are highly heterogeneous, and the immune environment within these tumours is often more deficient than that of peripheral tissue tumours. This leads to differences in the formation and function of TLSs in CNS tumours. These variations are particularly relevant in the context of glioma immunotherapy and could have important implications for treatment strategies. This review focuses on the composition and function of TLSs, examines the complexity of the glioblastoma (GBM) immune microenvironment, and highlights the unique characteristics of TLSs in GBM, providing new theoretical insights and practical foundations for targeting TLSs in glioma immunotherapy.

Keywords Tertiary lymphoid structure, Gliomas, Immunotherapy

Introduction

Gliomas, particularly glioblastomas (GBMs), represent a major challenge in neuro-oncology. Despite advances in diagnostic techniques and treatment modalities, the prognosis of glioma patients remains dismal, as the 5-year survival rate is less than 10% for GBM patients [1]. The immunosuppressive tumour microenvironment, characterized by a lack of effective immune surveillance and a highly immunosuppressive tumour-associated stroma, plays a pivotal role in facilitating tumour growth and therapeutic resistance.

In recent years, the presence of tertiary lymphoid structures (TLSs) in various solid tumours, including gliomas, has garnered increasing interest. TLSs are organized, ectopic lymphoid aggregates that form in response to chronic inflammation [2] and have been identified in several malignancies, including breast, lung, and colon cancers [3–5]. TLSs have been shown to form

†Jiatong Chen, Yuechao Yang and Shuang Luan are co-first authors.

*Correspondence:
Wenhao Xu
xwhao0407@163.com
Yang Gao
dryanggao@126.com
[1]Department of Neurosurgery, Fudan University Shanghai Cancer Center, Shanghai, China
[2]Department of Urology, Fudan University Shanghai Cancer Center, Shanghai, China
[3]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China
[4]Maternity & Child Care Center Of DeZhou, Shanghai, Shandong, China
[5]Shanghai Medical College, Fudan University, Shanghai, China

within the glioma microenvironment. The role of TLSs in gliomas remains controversial, as some evidence suggests that these structures can either enhance or inhibit immune-mediated tumour control. The formation of TLSs helps recruit T cells and other immune cells to promote an anti-tumour immune response, thereby inhibiting tumour growth. High endothelial venules (HEVs) and active lymphocyte infiltration in these structures may play key roles in an effective tumour immune response. However, TLSs may also provide shelter during tumour immune escape through local immune tolerance or they may promote immune escape mechanisms. Tumour cells may inhibit effective antitumour immune responses by modulating immune cell function within TLSs, particularly by inducing T-cell failure or promoting the accumulation of regulatory T cells (Tregs) [6]. Studying the formation and function of TLSs in gliomas may offer novel insights into the complex interplay between the immune system and glioma cells, potentially revealing new avenues for therapeutic intervention.

Structure and formation of TLSs

TLSs do not exist under normal physiological conditions; instead, they develop in specific pathological environments, such as in autoimmune diseases, during graft rejection and chronic inflammation, and in cancer [7]. These structures are organized in a way that mimics secondary lymphoid organs (SLOs), such as lymph nodes. The anatomical structure and function of TLSs are closely related to those of SLOs, such as lymph nodes [8]. However, TLSs lack the fibrous capsule that typically surrounds SLOs. This absence of a fibrous capsule enables immune cells within TLSs to interact directly with tumour tissue, thereby facilitating the rapid and efficient generation of anti-tumour immune responses [9].

TLSs are characterized by the presence of CD20 + B cells surrounded by CD3 + T cells, similar to the lymphoid follicles of SLOs [10]. The B lymphocyte subsets include plasma cells (PCs), regulatory B cells (Bregs) and memory B cells. The main subsets of T cells include CD4 + follicular helper T cells (Tfhs), CD8 + cytotoxic T cells, CD4 + T helper 1 (Th1) cells and Tregs. Other cells in the immune microenvironment also play crucial roles in the formation of TLSs. Macrophages, particularly proinflammatory M1 macrophages, contribute to the formation of TLSs by producing inflammatory cytokines. Stromal cells, including fibroblasts, endothelial cells and follicular dendritic cells (FDCs), are critical for the formation of TLSs [11]. These cells establish a favourable microenvironment for the formation of TLSs, which promotes the accumulation and functional activation of immune cells. Dendritic cells (DCs), natural killer cells, innate lymphoid cells, and neutrophils [2] may also be present. Peripheral node addressin (PNAd)-positive HEVs form a specialized vasculature for TLSs and promote lymphocyte recruitment [10].

SLOs, including the lymph nodes, spleen, tonsils, Peyer's patches, and mucosa-associated lymphoid tissue, are found throughout the body and can collect antigens from different tissues, thus promoting the induction of adaptive immune responses. In the case of persistent chronic inflammation, extranodal seeding of lymphatic tissue occurs, which leads to the formation of TLSs at the organ site [12]. To understand the way in which TLSs are formed, this process can be compared with the formation of SLOs.

The formation of SLOs requires a progressive interaction between the lymphoid tissue organizer (LTo) and the haematopoietic lymphoid tissue inducer (LTi). LTi cells are a type of innate lymphoid cell (ILC) derived from fetal liver hematopoietic stem cells that express the transcription factors RORγt and Id2. LTo cells are mesenchymal-derived stromal cells located at sites destined to form SLOs [10]. During embryogenesis, LTi cells are initially recruited to the site of scheduled lymph nodes by lymphatic endothelial cells (LECs) or mesenchymal cells expressing CCL21 [13]. Lymphotoxin, a member of the TNF superfamily, plays a pivotal role in lymphoid tissue development and exists primarily in two forms: lymphotoxin-α (LTα) and lymphotoxin-β (LTβ). When co-expressed with LTβ, LTα forms a cell surface-bound heterotrimer LTα1β2 that exclusively binds to the LTβ receptor (LTβR). LTi cells bind to their respective receptors on LTo cells via Lα1β2 and TNF [14]. This interaction triggers the NF-κB pathway [2], up-regulating the expression of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), mucosal addressin cell-adhesion molecule 1 (MAdCAM1) and PNAd, as well as the production of a group of lymphoid chemokines, including CCL19, CCL21, and CXCL13 [10]. These molecules regulate the recruitment of immune cells and the vascularization of HEVs. Moreover, the secretion of lymphoid chemokines also induces a positive feedback pathway, which leads to the recruitment of more LTi cells [15]. In addition to LTα1β2, the LTβR also binds LIGHT as its ligand. In TLSs, the LIGHT-LTβR signaling pathway primarily induces the formation of high endothelial venules HEVs and facilitates lymphocyte migration [16]. Consequently, many researchers consider LIGHT a potential tumor immune target that can target specific receptors on tumor vasculature to promote TLSs formation [17].

During TLS formation, the definitions of LTi cells and LTo cells are not as strict as they are during SLO formation (Fig. 1). T helper 17 (Th17) cells, ILC3 cells, CD8 + T cells, B cells, and M1-polarized macrophages can serve as LTi cells, whereas fibroblasts, adipocytes, and vascular smooth muscle cells in the tumour microenvironment

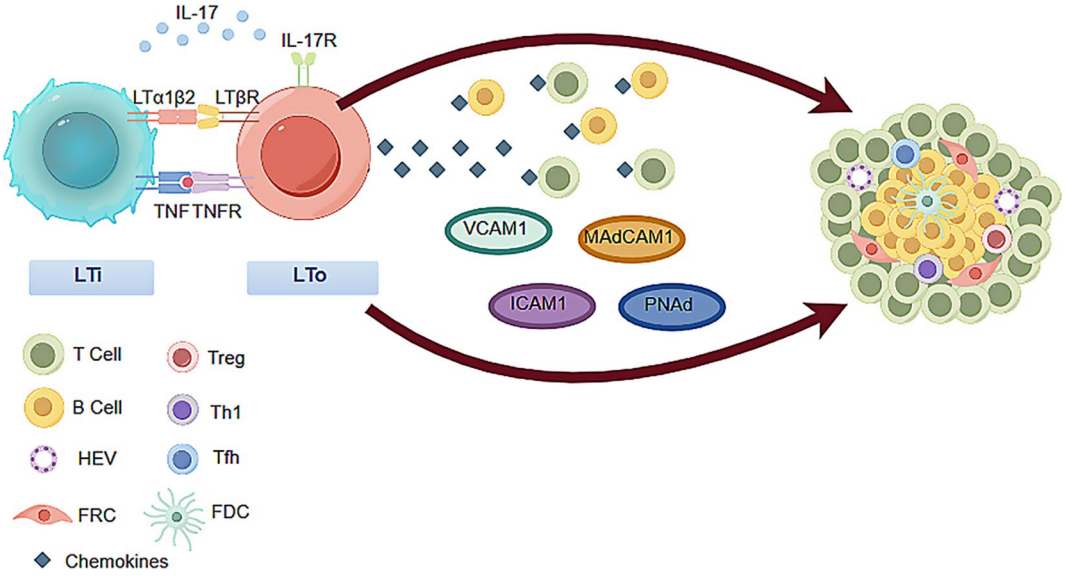

Fig. 1 Interaction of LTo and LTi

can act as LTo cells [11, 18–23]. Moreover, the induction of TLSs may not always be dependent on lymphoids, for example, T cell-produced IL-17 can induce mouse stromal cells to express CXCL13 and CCL19, thus promoting the induction of broncho-associated lymphoid tissue (iBALT) formation, a type of TLSs that is formed in lung tissue [24].

Chemokines play an important role in the formation of TLSs. They form a strong local chemical gradient that attracts lymphocytes from neighboring HEVs and precisely regulates their entry into T cell and B cell regions. This spatial separation depends on the differential distribution of chemokines, forming a well-defined T/B cell partition [25]. CCL19/CCL21 and CXCL13 are the most important chemokines. CCL19/CCL21 binds to CCR7, produced mainly by stromal and epithelial cells, and can attract T cells and antigen-stimulated DCs, promoting the development of T cell regions in TLSs [26]. In triple-negative breast cancer, the presence of CCL19 + DCs is associated with TLSs and T cell aggregation [27]. In addition to its role in inducing chemotactic migration, CCL21 also acts as a co-stimulatory factor for CD4 + and CD8 + T cell expansion and induces Th1 polarization [28]. Anti-tumor effector NK and NKT cell subsets also express the CCR7 receptor and are chemically attracted to CCL21. In conclusion, CCL19/CCL21 has a powerful anti-tumour immune effect and is highly important in cancer treatment. CXCL13 recruits B cells by selectively binding to CXCR5. Initially, activated stromal cells, particularly fibroblast reticular cells (FRCs), are the primary source of CXCL13 during the early stages of TLSs development [29]. Subsequently, macrophages, DCs, FDCs, and T cells can also produce CXCL13. After the construction of FDCs network, FDCs become the main cell producing CXCL13 in germinal centres (GCs) [2]. Recruited B cells also produce CXCL13, which creates a positive feedback loop that is important for lymphocyte aggregation, GC formation, and antibody production. CXCL13 can also exhibit antitumor effects by recruiting, activating, and expanding CXCR5 + CD8 + T cells [30]. However, high levels of CXCL13 do not always predict a good prognosis. Recent studies have shown that infiltration of CD8 + T cells with high CXCL13 expression in renal carcinoma leads to an immunosuppressive microenvironment [31, 32].

Although numerous studies have identified factors influencing TLSs formation within the tumour microenvironment, our understanding of the specific molecular in the local environment that either promote or inhibit TLS development remains incomplete. Moreover, the induction mechanism of TLSs in different tumors is still unclear, and a unified model is lacking. Addressing these limitations will be pivotal in advancing TLSs research from biological discovery to clinical translation.

The heterogeneity of TLSs in cancer

In recent years, with further elucidation of the composition and function of TLSs, the prognostic value of these structures in various solid tumours has gradually become clearer. TLSs exhibit significant heterogeneity, which is reflected in noticeable differences in the density of TLSs, their distribution within the tumour, and their maturity, both between patients with the same type of cancer and those with different cancer types. These factors are closely associated with patient prognosis.

Density of TLSs

The density of TLSs is associated with the formation of GCs and the expression of genes involved in adaptive immune responses, thus, the density is an independent prognostic marker for some tumours. For example, one study that reviewed 1,033 patients with gastric cancer who underwent gastrectomy revealed that high levels of TLSs were significantly associated with tumour size ($p=.047$), histological grade ($p=.039$), pTN stage ($p=.044$), and World Health Organization (WHO) subtype ($p<.001$), which suggests that high TLS density is associated with a favourable prognosis among patients with gastric cancer [33]. Another study of lung squamous cell carcinoma confirmed that TLS density was the strongest independent prognostic marker in untreated patients and that TLS density was associated with the expression of genes associated with GC formation and the adaptive immune response [34]. However, in patients who receive neoadjuvant chemotherapy, the TLS density is similar, but GC formation is impaired, and the prognostic value of TLS density is lost, which suggests that GC formation is strongly associated with a favourable prognosis. In addition, TLS density and GC formation are independent prognostic markers in both colorectal [35] and pancreatic cancers [36].

Location of TLSs

The relative spatial positioning of TLSs within the tumour may also influence their mechanism of action and potentially affect patient prognosis [37]. With respect to their location, when TLSs are located within the tumour, cancer patients tend to have a better prognosis. A study of intrahepatic cholangiocarcinoma that included 962 patients was performed to establish a TLS scoring system for the intratumoral area (T score) and peritumour area (P score) [38]. The results revealed that the T score was positively correlated with favourable prognosis ($p<.001$). In another study of intrahepatic cholangiocarcinoma, samples with a high T score had a smaller tumour diameter than did those with a low T score ($p=.010$) [39]. However, when TLSs are located around a tumour, strong heterogeneity is observed in prognosis. In a study of 114 colorectal cancer (CRC) patients, low peritumoral TLS (P-TLS) density ($<0.098/mm^2$) was associated with reduced relapse-free survival (RFS) in non-metastatic colorectal cancer (nmCRC) patients (HR = 6.597 95% CI: 2.882–15.103, $p<.001$) and lower overall survival (OS) (HR = 6.628 95% CI: 2.893–15.183, $p<.001$) [4]. These findings suggest that high P-TLS density is an independent and favourable prognostic factor for nmCRC patients and may provide a new direction for targeted therapy for CRC. According to one study, in cholangiocarcinoma, a high P score was negatively associated with a good prognosis ($p<.001$) [38]. Patients with high P scores had a greater incidence of satellite lesions ($p=.048$) and lymphatic metastases ($p=.040$) than did patients with low P scores, and higher P scores were also found to be positively associated with fatty liver disease ($p=.022$) [39]. The proportions of both T follicular helper cells and regulatory T cells were significantly greater in intratumoral TLSs than in peritumoral TLSs ($p<.05$), but the percentage of regulatory T cells in intratumoral TLSs was positively correlated with the P score ($p<.05$) but not the T score [38]. In summary, intratumoral TLSs are associated with a good prognosis, while peritumoral TLSs are associated with some prognostic heterogeneity.

Maturity of TLSs

With respect to the maturity of TLSs, Posch et al. [35] proposed that the development of tumour-associated TLSs involves three stages. The first stage involves early TLSs (CD21-CD23-) containing T cells, B cells, and perivascular cells that express CXCL13, with no FDCs or GCs. The second stage involves primary follicle-like TLSs (CD21 + CD23-) that contain HEVs and FDCs but not GCs. Finally, the third stage involves secondary follicle-like TLSs (CD21 + CD23+) that are more mature and contain HEVs, FDCs, and GCs, resembling SLOs. Meylan et al. reported that in precancerous lesions of the liver, early TLSs lacking GCs may favour immune evasion by tumour cells and promote the progression of precancerous lesions to mature hepatocellular carcinoma [40]. In a study of lung adenocarcinoma, researchers analysed 218 patients who underwent radical resection for lung adenocarcinoma [41]. TLSs were divided into a high-maturity group (high DC-Lamp group) and a low-maturity group (low DC-Lamp group) according to immunohistochemistry results, and mature TLSs were confirmed to be associated with an increased number of cytotoxic T lymphocytes (CTLs) in draining lymph nodes (20.0 vs. 15.1, $p=.017$) and a decreased frequency of lymphocyte metastasis ($p<.0001$). Mature TLSs were also independently associated with good overall survival (HR = 0.17, $p=.0220$) and disease-free survival (HR = 0.54, $p=.0436$). These findings suggest that mature TLSs may enhance the antitumour effects of immunotherapy by activating lymphocytes and increasing the number of CTLs in the draining lymph nodes.

In general, the density, location, and maturity of TLSs vary greatly among individuals, and this high degree of heterogeneity requires better classification and research for clinical application.

TLS in autoimmune diseases of the CNS

As mentioned earlier, TLSs can also appear in autoimmune diseases. Comparing TLSs in autoimmune diseases of the central nervous system with glioma provides

insight into their development and function in the brain cancer environment.

Multiple sclerosis (MS)

MS is a neuroinflammatory autoimmune disease. In a mouse model of MS, the presence of TLSs was associated with an increased proportion of meningeal PDPN + PDGFRα + PDGFRβ + fibroblastic reticular cells, which express the LTβR and CXCL13 [42]. In patients with secondary progressive multiple sclerosis (SPMS), TLS have been identified in the meninges of approximately 40% of cases [6]. These structures contain B cell follicles, T cell zones, and FDC networks, and are closely associated with cortical pathology and the degree of disability. Studies have found a significant presence of activated Tfh1 cells in the cerebrospinal fluid (CSF) of MS patients. These cells exhibit high migratory capacity, enabling them to cross the blood-brain barrier and enter the CNS. Tfh cells play a crucial role in the formation and maintenance of TLS. Tfh1 cells in the CSF produce cytokines such as IFN-γ, which support the differentiation of B cells into antibody-secreting cells (ASCs) and promote the production of autoantibodies, playing an important role in the pathological process of MS [43]. Additionally, Tfh cells show up-regulation of cytotoxic genes, such as Granzyme A (GZMA), Granzyme H (GZMH), Granzyme K (GZMK), and Perforin 1 (PRF1), suggesting that Tfh cells may possess cytotoxic functions in MS [44]. This evidence highlights that TLSs exacerbate the characteristic autoreactive immune responses against CNS self-antigens, underscoring their potential as therapeutic targets.

Autoimmune uveitis

In related studies on human samples, TLS were found in approximately 20% of cases, with the uvea being the primary ocular tissue affected by immune cell infiltration, while only sparse lymphocytes were visible in the retina [45]. However, in mouse models of uveitis, TLS-like structures are predominantly located in the retina. This discrepancy may be related to differences in the anatomical structure of the choroid between mice and humans, or it may be associated with the chronicity of the disease. In mouse models of uveitis, TLS-like structures are composed of Tfh, B cells, plasmablasts, plasma cells, and antigen-presenting cells (APCs), with B cells being the dominant population. The formation of TLS is closely related to Th1 cell-driven immune responses. Th1 cells play a central role in the disease by producing IFN-γ. IFN-γ not only affects immune cells but also influences Müller glial cells in the retina. Müller glial cells may act as APCs and directly interact with CD4 + T cells, further promoting local immune responses [46]. Interestingly, in the early stages of the disease, the presence of TLS is associated with lower clinical and histological disease scores and slower loss of visual function compared to retinas without TLS. However, as TLS mature and the number of plasma cells increases, TLS become more diffuse and disorganized, leading to accelerated visual function loss and disease progression in mice [47]. This suggests that TLS may not uniformly function to promote autoimmune diseases, and well-organized, functional TLS may hold autoimmune attacks at bay.

Neuropsychiatric lupus erythematosus (NPSLE)

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that affects multiple organs. The manifestations of NPSLE are diverse, including cognitive dysfunction, mood disorders, and more, but its pathogenesis remains poorly understood. The choroid plexus is an important immune interface in the CNS, responsible for the production of CSF and serving as a gateway for immune cells to enter the CNS under inflammatory conditions. In mouse models, significant infiltration of B cells and T cells is observed in the choroid plexus, forming structures similar to TLS with germinal center GC activity. The TLS in the choroid plexus are likely sites for the local production of auto-antibodies, directly contributing to neuropsychiatric symptoms and driving the progression of NPSLE [48]. Additionally, it has been found that choroid plexus epithelial cells transport lymphocytes from the stroma into the ventricles through trans-epithelial migration, further supporting the critical role of TLS in NPSLE [49].

Tumour-associated lymphoid structures in glioma

Glioma

Glioma is the most common primary malignant tumour of the CNS in adults, and of all gliomas, grade IV GBM has the highest incidence and accounts for 50.1% of all primary malignant CNS tumours. Despite various treatment options, such as surgical resection, radiotherapy, and chemotherapy, the survival time of GBM patients remains short. Studies have shown that the median overall survival (mOS) of GBM patients after diagnosis is less than one year. The first-line treatment regimen STUPP can extend the mOS to 16 months, and when the STUPP regimen is combined with tumour treatment fields (TTFs), the mOS increases to 20.9 months [50, 51]. Immune checkpoint inhibitors (ICIs) have been shown to be effective in several solid tumours, but GBM patients have not benefited from this breakthrough [52]. Adjuvant α-PD-1 therapy has not yet been demonstrated to be effective for gliomas, and neoadjuvant α-PD-1 therapy only slightly increases immune activation [53]. The main reason for this is that the effectiveness of ICIs strictly depends on preexisting T-cell responses to the tumour, but the TIME in the CNS lacks tumour antigen-specific T cells. Therefore, a deeper understanding of the

mechanisms underlying the involvement of TLSs in gliomas will be beneficial for the clinical application of glioma immunotherapy.

The immune microenvironment of GBM

The immune microenvironment of GBM is highly complex and plays a pivotal role in tumour progression, response to therapy, and overall prognosis. According to the degree of CTL infiltration, the tumour phenotype can be classified as an "immune-desert", "immune-excluded" or "immune-inflamed" phenotype. Tumours with an immune-inflammatory phenotype are more likely to respond to ICI treatment [54]. Despite the presence of immune cells such as TILs, macrophages, and microglia [55], GBM tumours tend to establish an immunosuppressive microenvironment that hampers effective antitumour immune responses, resulting in an "immune-desert" phenotype. This means that immune cells in the GBM tumour microenvironment are unable to play an effective immune role compared with immune cells in other tumour types.

This immune escape is driven by numerous factors. GBM induces systemic immunosuppression by down-regulating sphingosine-1-phosphate receptor 1 (S1P1) and isolating naive T cells in the bone marrow [56]. In addition, blood flow during the neovascularization process in GBM is slow and irregular, and many dysfunctional microvessels develop. This results in a hypoxic tumour microenvironment, which affects immune cell function [57]. Many immunosuppressive cells, such as tumour-associated macrophages and microglia, produce low levels of pro-inflammatory cytokines and lack key molecules involved in T cell costimulation (CD86, CD80, and CD40) [58]. Tregs are a subgroup of T cells. GBMs can express indoleamine 2,3-dioxygenase 1 (IDO1), IL-10, C-C motif chemokine ligand 2 (CCL2) and transforming growth factor β (TGF-β), which promote the expansion of Tregs in the TIME, leading to the dysfunction and depletion of TILs [59]. Additionally, the blood–brain barrier (BBB) poses a significant challenge for immune cell infiltration and therapeutic intervention [60]. Understanding the intricate interactions within the GBM immune microenvironment is crucial for the development of effective immunotherapies and treatment strategies.

Characteristics of TLS formation in glioma

The formation of TLS in gliomas is limited by the unique anatomical and immune environment of the central nervous system, characterized by low frequency, immature structure, and immunosuppressive cell infiltration, and is therefore relatively rare in gliomas, in contrast to functional TLS in other tumors. Although it is difficult for circulating immune cells and antibodies in a static state to penetrate the BBB, when a danger signal is detected, peripheral immune cells can cross the BBB and induce a severe inflammatory response, providing the basis for immunotherapy in brain tumours [60]. In GBM, due to increased angiogenesis and the release of cytokines and chemical mediators, the BBB is partially destroyed, and consequently, its permeability is increased [61]. Specific interactions between glioma cells and the vascular system allow immune cells to extravasate and promote the formation of TLSs at the tumour site. Moreover, some of the processes involved in establishing an immunosuppressive microenvironment can be artificially reversed, which may improve the immune microenvironment of the CNS and increase the tumour response to immunotherapy. Therefore, TLSs are highly important in the treatment of glioma.

The TLSs in mouse models of glioma are similar to those found in peripheral tumours, but they are uniquely encased within extracellular matrix molecules, such as collagen and fibronectin, and exhibit an elongated morphology (Fig. 2). In humans, TLSs are found mainly in WHO grade II-IV gliomas. While it was previously believed that TLSs are present only in the meningeal region, recent studies have shown that TLSs can also form within the white matter area near the tumour and within the tumour tissue itself, and around the PNAd+ HEVs, suggesting that the mechanism of TLSs formation in brain cancer may be different from that in autoimmune diseases [6].

In the past, the brain was considered an immune privileged organ. However, recent studies have shown that the brain is actually an active immune monitoring point maintained by the meningeal lymphatic system and that CNS antigens can be drained from the cerebrospinal fluid into the cervical lymph nodes [62]. Specifically, CSF enters the brain in the periarterial space, enters the interstitium via aquaporin 4, and exits through the perivenous space into the deep cervical and lumbar lymph nodes [63]. Immune cells can enter the CNS through soft meningeal blood vessels and the highly vascularized choroidal plexus from the subarachnoid space. The meninges are rich in immune cells and contain postcapillary venules that support immune cell migration [64], and immune responses in the CNS are typically initiated in the meninges. This may explain why TLSs in the CNS are more often found in the meninges or choroid plexus than within the parenchyma. In the cortex, TLS usually surrounds HEVs. The formation of HEVs is associated with sustained immune responses and can be enhanced by the depletion of Treg cells in peripheral tumours [65]. Therefore, the presence of HEVs may allow TLSs to form outside the meninges. Another possibility is that cortical TLSs are directly connected to meningeal tissue through the Virchow–Robin space [66].

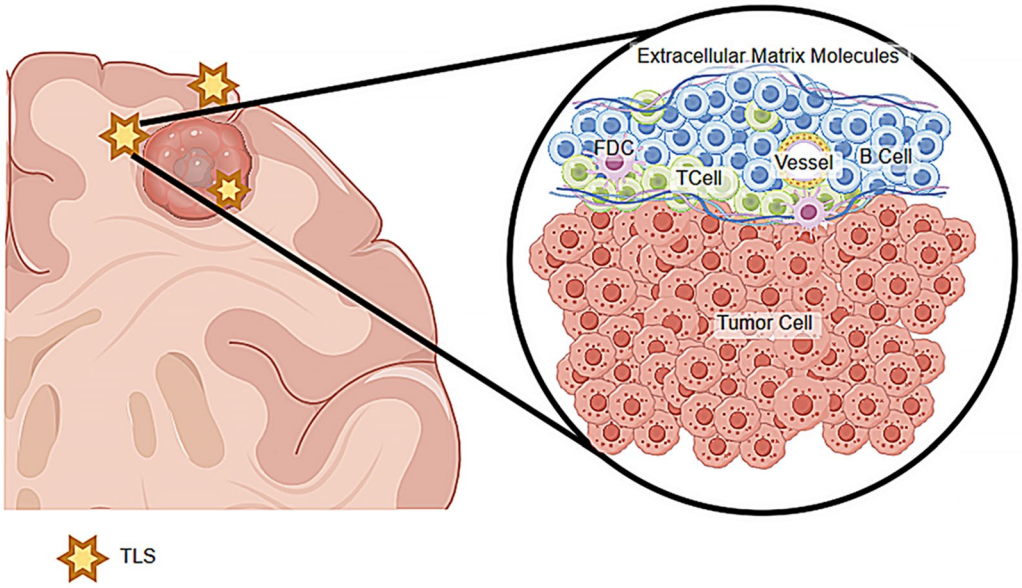

Fig. 2 Location and morphology of TLSs in mouse glioma

Table 1 Key differences in TLSs between gliomas and peripheral tumours

Features	TLSs in gliomas	TLSs in perpheral tumours
Location	Predominantly at tumour margins/peritumoral areas, influenced by BBB	Distributed in tumour core/stroma, often near vasculature
Maturity	Immature TLSs, poorly defined B cell/T cell zones	More mature TLSs with GC and clear zoning
Immune composition	B cell and Treg dominance; fewer CD8 + T cells	Balanced B cells, CD4+/CD8 + T cells, DCs
Functional role	Immunosuppressive	Pro-inflammatory, supports antitumor immunity
Lymphatic link	Lack of functional lymphatic vessels, rely on perivascular spaces	Co-located with new lymphatic vessels for cell trafficking
Therapeutic response	Poor response to ICIs	ICIs may enhance TLS-mediated immunity

Thus far, it is unclear which cells initiate and maintain the development of TLSs in the CNS. Research suggests that in the formation of glioma, TLSs may be related to stromal cells in the CNS, including fibroblasts, LECs, blood endothelial cells, pericytes, and choroid plexus epithelial cells, all of which are distinctly distributed in specific stromal niches [67]. However, the potential roles of these stromal cells in the formation of glioma TLSs and the mechanisms underlying these roles have not been investigated.

In conclusion, TLS in gliomas exhibit marked differences compared to those in perpheral tumours (Table 1). A thorough comparison of these distinctions not only enhances our understanding of the unique mechanisms underlying TLS formation within the CNS but also paves the way for novel therapeutic strategies aimed at inducing TLS development.

Heterogeneity of TLSs in gliomas

The role of TLS in gliomas is relatively complex. Zhou et al. used consensus cluster analysis of TLS gene expression profiles from the cancer genome atlas (TCGA) to classify gliomas into three subtypes: A, B and C. Among them, patients with the C subtype have a shorter survival period, poorer prognosis, and are associated with malignant features such as high-grade gliomas, isocitrate dehydrogenase (IDH) wild-type, and anaplastic glioma. At the cellular level, gliomas of subtype C have a higher level of immune invasion, especially the number of anti-tumor immune cells such as B cells, T cells and natural killer cells is significantly increased. However, there are also more immunosuppressive cells in subtype C such as M2 macrophages, regulatory T cells, which may weaken the anti-tumor effect of TLSs. In addition, subtype C shows a higher sensitivity to multiple drugs, while subtype A is more sensitive to certain medications, such as paclitaxel [68]. This typing revealed the heterogeneity of TLSs in gliomas.

It provides a new perspective on immunotherapy of gliomas. Although TLS has shown a good prognostic correlation in a variety of cancers, in gliomas, its role may be regulated by the immunosuppressive microenvironment. Future research should focus on optimizing immunotherapy strategies by regulating the formation of TLSs, especially its potential application in immunologically "cold" tumors.

TLSs in the clinical treatment of gliomas: applications and limitations

Effects of radiotherapy and chemotherapy on glioma TLSs

Radiotherapy and chemotherapy are critical aspects of standard treatments for gliomas. These therapies can influence the TIME, including TLSs, in complex ways. For example, the direct injection of chemotherapy drugs into the GBM site can lead to tumour recurrence and drug resistance. This approach fails to effectively target the malignant cells infiltrating the tumour and does not account for the inherent heterogeneity of the tumour [69]. Radiotherapy also has an inhibitory effect on activated B cells and PCs [70]. Neoadjuvant therapy can reduce the primary tumour load, induce a persistent antitumour immune response, and eliminate small residual disease, offering patients the ability to undergo surgery. In a non-small cell lung cancer (NSCLC) study [71], neoadjuvant chemotherapy was associated with increased infiltration of cytotoxic T cells and B cells and reduced density of forkhead box P3 (FoxP3) cells, which indicates a positive effect on TLS formation. However, research on neoadjuvant therapy for gliomas is lacking. Understanding the effects of these therapies on TLSs could help guide the development of combined treatment strategies that not only target tumour cells but also modulate the immune environment to enhance therapeutic outcomes.

TLSs and immunotherapy in gliomas

The immunological significance of TLSs in gliomas is multifaceted, as emerging evidence suggests both protumour and anti-tumour effects. TLS formation represents an attempt by the immune system to counteract tumour growth, but the tumour microenvironment can also exploit TLSs to promote tumour progression. The presence of glioma-associated TLSs suggests that these structures can serve as alternative sites for antigen presentation and T-cell activation. As TLSs are sites involved in controlling tumour progression and generating circulating immune cells, their TLS function does not rely on programmed death-ligand 1 (PD-L1) expression [72], thus, they show great promise as therapeutic targets. Since the formation of TLSs is a dynamic process that can be manipulated, this process provides an exciting new target for enhancing the activation of tumour-reactive T cells in gliomas.

Several studies have been conducted to provide potential targeted treatment strategies for gliomas by inducing the formation of TLS. Dimberg et al. [17] found that targeting vascular endothelial cells in the tumor microenvironment can regulate the function of TLSs. They selected LIGHT from a group of molecules associated with lymphocyte generation as the most promising therapeutic candidate for GBM. The researchers used an adeno-associated viral (AAV) vector targeting brain endothelial cells to express LIGHT (AAV-LIGHT) in the glioma vasculature and reported that systemic AAV-LIGHT treatment induced the formation of tumour-associated HEVs and T-cell-rich TLSs, thereby extending the survival of α-PD-1-resistant mice with gliomas. The mechanism behind this effect is that AAV-LIGHT treatment reduces T-cell exhaustion and promotes the generation of TCF1 + CD8 + stem cell-like T cells. By targeting the vasculature to express LIGHT, the vascular phenotype is altered, which promotes effective anti-tumour T-cell responses and extends the survival of glioma-bearing mice (Fig. 3). Toll-like receptor (TLR) can recognise pathogen-associated molecular patterns (PAMPs) and are a crucial component of the immune system, protecting the host from pathogen infections. Additionally, TLR serves as one of the links between innate immunity and adaptive immunity [73]. Shen et al. [74] successfully induced the formation of TLSs in the glioma microenvironment via the intracranial injection of TLR agonists and glioma antigens (α-TLR-mix) into mice. This approach increased lymphocyte infiltration into the immune microenvironment and improved the prognosis of the mice. During this process, certain macrophages/microglia and Th17 cells express LTo and LTi cell markers, respectively, which promote TLS formation through their interactions. Further analysis of the immune cell components revealed that CD4 + T cells play a more critical role in anti-glioma immunity, while CD8 + T cells also contribute significantly. In contrast, CD19 + B cells did not exhibit a notable anti-tumour effect. However, B cells were found to be located within or around TLSs and were almost entirely absent in normal brain samples, suggesting their potential as a target for investigating the function of TLSs in the glioma microenvironment. Clinical studies demonstrated that α-TLR-mix treatment was well-tolerated in patients with recurrent GBM and significantly prolonged progression-free survival (PFS) and OS compared to the control group. Human cytomegalovirus (HCMV) immediate early protein 1 (IE1) is highly expressed in GBM tissues and is closely associated with the malignant progression of the tumour [75]. Yang et al. [76] developed a therapeutic vaccine targeting HCMV IE1 and its mutant form, IE1mut, and conducted experiments in mouse glioma models. The results demonstrated that the IE1/IE1mut vaccine significantly reduced tumour size and improved the survival rate of the mice. The vaccine functions by promoting the formation of TLSs within the tumour and activating the peripheral immune system. A large number of proliferating T cells were observed in the treated group, with CTLs partially polarising into effector memory T cells and being activated in peripheral immune organs. B cells in the draining lymph nodes also exhibited high expression of CD40 and CD86. Furthermore, the IE1mut vaccine,

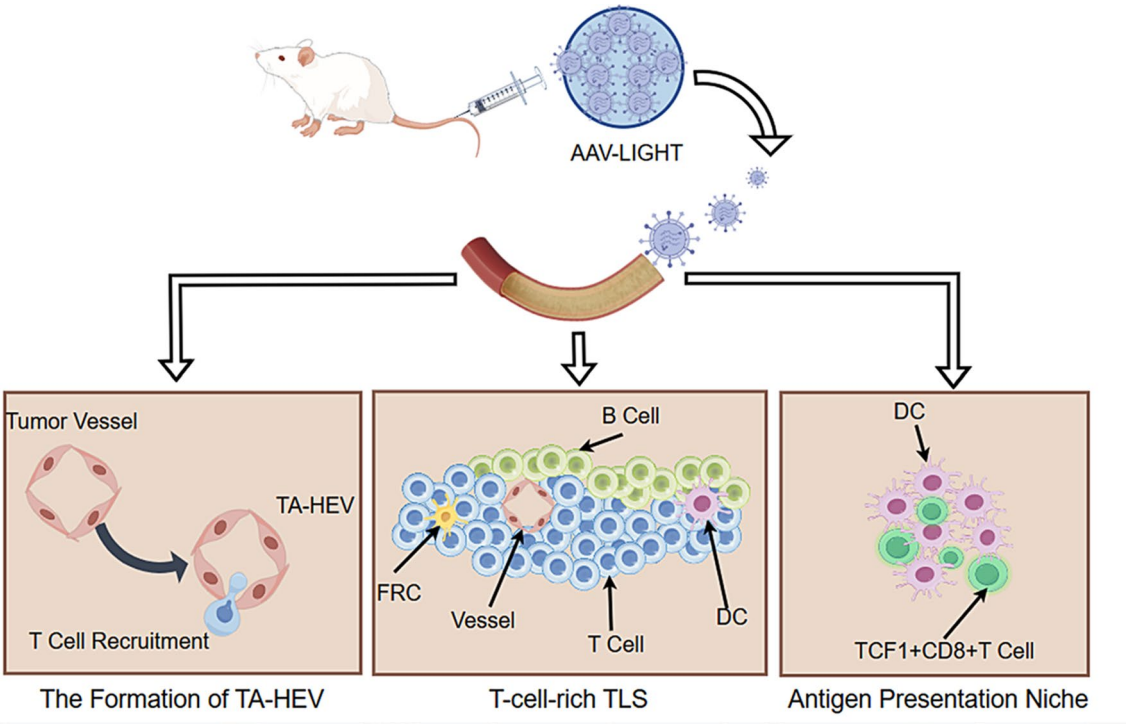

Fig. 3 AAV-LIGHT treatment for GBM

while reducing the potential toxicity of IE1, retained the same immunotherapeutic efficacy as IE1. These findings not only reveal the key role of TLS in the glioma immune microenvironment, but also provide an important theoretical basis for the future development of TLS-based induction immunotherapy strategies with important clinical translational potential.

In addition to ongoing experiments, some researchers have innovatively proposed combining inducible tertiary lymphoid structures (iTLS) with chimeric antigen receptor T (CAR-T) cells and stimulator of interferon genes (STING) agonists, providing new insights for the immunotherapy of GBM (Fig. 4) [77]. CAR-T cells directly kill tumor cells by recognizing tumor-associated antigens (TAA), but their persistence and efficacy in solid tumors are limited by the tumor microenvironment [78]. By locally delivering CAR-T cells and combining them with the formation of TLS, the infiltration and activation of T cells can be enhanced, thereby overcoming the immunosuppressive tumor microenvironment. STING agonists further amplify the anti-tumor immune response by activating the type I interferon pathway [79]. Additionally, glioma stem cells (GSCs), which are the primary source of tumor recurrence and drug resistance [80], have also been incorporated into local immunotherapy strategies. By targeting GSCs, CAR-T cells can more effectively eliminate tumor cells and reduce the risk of recurrence. To efficiently deliver these immunotherapeutic components to the tumor site, biodegradable scaffolds have emerged as an ideal carrier. Scaffolds not only provide structural support but also enable controlled release of immunotherapeutic agents, maintaining a high local drug concentration and thereby enhancing therapeutic efficacy [81]. This innovative approach can induce TLS formation through the injection or implantation of biomaterials after tumor resection, helping to control residual disease and prevent recurrence. Despite the significant potential of iTLS in GBM treatment, its clinical application still faces numerous challenges. First, the biocompatibility and degradability of scaffold materials need further optimization to avoid foreign body reactions (FBR) and immune rejection [81]. Second, the formation and function of iTLS are influenced by various factors in the tumour microenvironment, such as inflammatory factors, stromal cells, and angiogenesis, necessitating in-depth research into how these factors affect iTLS. Additionally, the long-term safety and potential side effects of iTLS need to be evaluated in clinical trials. With a deeper understanding of the mechanisms underlying TLS formation and immune regulation, iTLS holds promise as a crucial tool in GBM immunotherapy, offering new hope for patients.

CD40L (CD40 ligand, CD154) is expressed by activated CD4 + T cells and binds to CD40 on DCs and B cells, driving DC maturation, B cell activation, and antibody production [82]. As localised immunological hubs, TLSs are enriched with CD40 + APCs and CD40L + T cells. Elevated CD40 expression correlates with enhanced

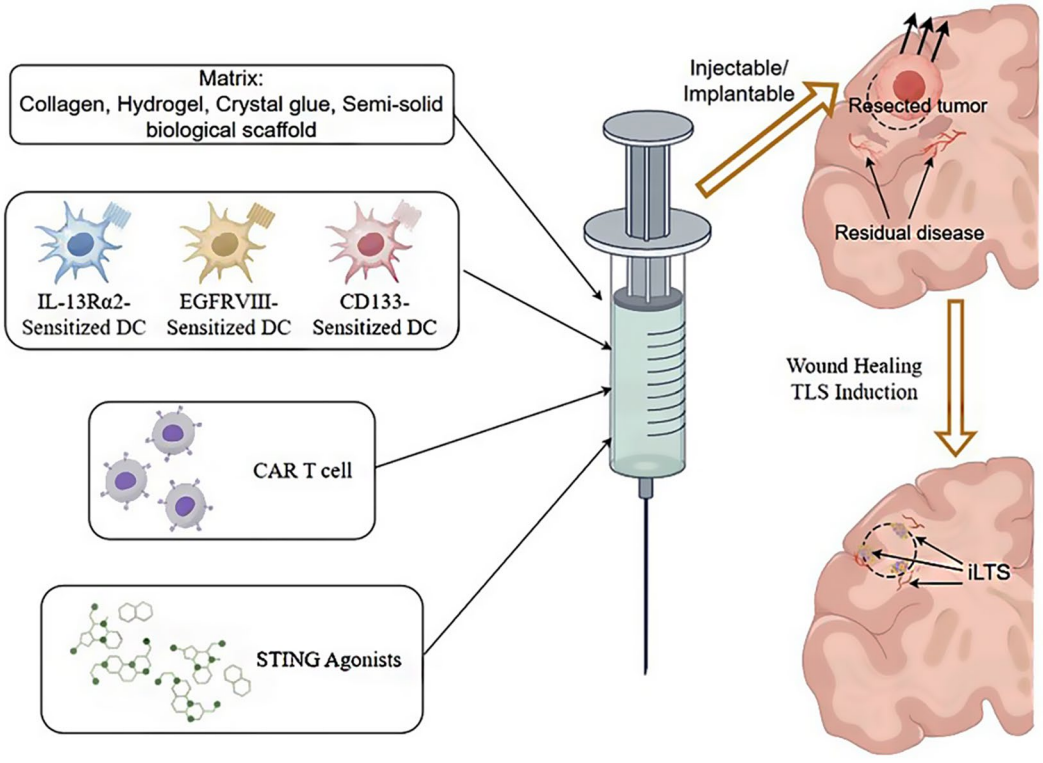

Fig. 4 Injection or implantation of iTLS

responses to ICI, positioning it as a potential predictive biomarker for immunotherapy efficacy [83, 84]. Immunostimulatory agonistic CD40 antibodies (αCD40) are currently under clinical development and have broad immunostimulatory effects, being used in the treatment of many solid tumors, but they have not yet been evaluated for gliomas [85]. Preclinical glioma models have shown that systemic administration of αCD40 leads to the development of TLS near meningeal tissue. By stimulating B cells to up-regulate LTα expression, αCD40 promotes the formation of TLS. Additionally, αCD40 can increase T cell infiltration, but these T cells are hypo-functional and exhibit reduced responsiveness to immune checkpoint inhibitors. Analysis has revealed that these phenomena are associated with the expansion of suppressive CD11b + B cells, which may inhibit T cell responses through cell-to-cell interactions. Further research has found that the expression of CD11b is not directly induced by αCD40 stimulation of B cells but is instead related to a systemic increase in IL-10 [86]. A recent study demonstrated that 4-1BBL + B cells activated in vitro with αCD40 and IFN-γ elicited antitumor immunity in glioma-bearing mice [87]. This suggests that by activating B cells ex vivo, it is possible to bypass the up-regulation of CD11b while enhancing antitumor immune responses. In summary, the pleiotropic effects of αCD40 therapy in gliomas highlight the need for further research to explore the importance of TLS in immunotherapy responses and how to modulate TLS to either enhance or suppress immune reactions.

It is also important to note that strong immune activation within the central nervous system during immunotherapy is associated with certain risks, including edema and autoimmune reactions [6]. CNS oedema, which is constrained by the skull, can have destructive effects, such as increasing intracranial pressure, impairing function, and even causing death. Additionally, immune therapies targeting TLSs formed in the meninges may lead to the local activation of autoreactive lymphocytes, which can attack normal neural tissue, similar to MS [88]. Corticosteroids can be used to alleviate symptoms of CNS oedema and autoimmune attacks, but they may also suppress the effectiveness of immunotherapy and TLS formation. Studies have shown that the use of dexamethasone in GBM patients receiving ICI therapy is associated with a shorter survival time [89]. Therefore, caution should be exercised when combining corticosteroid treatment with immunotherapy, as it may counteract TLS formation and reduce anti-tumour immune responses.

Breakthroughs and future perspectives of TLSs in glioma immunotherapy

Many methods for local GBM treatment have been studied. However, due to the immune-suppressive tumour microenvironment and complex immune escape

mechanisms in GBM, these treatments have either shown limited effectiveness or have been unsuccessful.

The discovery of TLSs in glioma is highly significant. Future research should focus on several issues to maximize the potential of local immunotherapy methods and to overcome the limitations of existing technologies. First, more research is needed to improve the understanding of the complex interplay between TLS-related immune responses in the GBM microenvironment. For example, what factors influence TLS formation and dynamic development in tumours, how can the composition, function, commonality and characteristics of TLSs in different cancers be comprehensively characterized, and are TLSs the cause or effect of tumour immunity? Second, the composition of the biomaterials that induce TLS formation should be optimized, and long-term safety and possible negative effects should be monitored to achieve better therapeutic outcomes. In addition, research that focuses on other therapeutic modalities, such as the combination of conventional drugs or novel immunomodulatory drugs, can improve the efficacy of treatment and overcome possible drug resistance mechanisms to reduce the risk of intracranial oedema and autoimmune diseases.

As more data emerge, the value of TLSs as therapeutic targets will become clearer. The integration of TLSs into routine clinical practice could usher in a new era of precision medicine for glioma immunotherapy.

Conclusions

TLSs in gliomas are an intriguing component of the tumour microenvironment and play a complex role in regulating immune responses against tumours. While TLSs have the potential to enhance anti-tumour immunity, they can also contribute to immune suppression and tumour progression. The study of TLSs in gliomas holds promise for the development of novel therapeutic strategies aimed at modulating the immune response to improve patient outcomes. However, further research is needed to fully understand the dynamics of TLSs in gliomas and their potential as therapeutic targets.

Abbreviations

TLSs	Tertiary lymphoid structures
TIME	Tumour immune microenvironment
TILs	Tumour-infiltrating immune cells
CNS	Central nervous system
GBM	Glioblastoma
HEVs	High endothelial venules
Tregs	Regulatory T cells
SLOs	Secondary lymphoid organs
PCs	Plasma cells
Breg	Regulatory B cells
Tfh	Follicular helper T cells
Th1	T helper 1 cell
FDCs	Follicular dendritic cells
DCs	Dendritic cells
PNAd	Peripheral node addressin
LTo	Lymphoid tissue organizer
LTi	Lymphoid tissue inducer
ID2	Inhibitor of DNA binding 2
ILC	Lymphoid cell
LECs	Lymphatic endothelial cells
LTα	Lymphotoxin-α
LTβ	Lymphotoxin-β
LTα1β2	Lymphophotoxin α1β2
LTβR	Lymphotoxin beta receptor
TNF	Tumour necrosis factor
VCAM1	Vascular celladhesion molecule 1
ICAM1	Intercellular adhesion molecule 1
MAdCAM1	Mucosal addressin cell-adhesion molecule 1
Th17	T helper 17 cell
iBALT	Induction of broncho-associated lymphoid tissue
FRCs	Fibroblast reticular cells
CCL19	Chemokine ligand 19
CCL21	Chemokine ligand 21
CXCL13	C-X-C motif chemokine ligand 13
GC	Geriatric centers
WHO	World Health Organization
CRC	Colorectal cancer
P-TLS	Peritumoral TLS
RFS	Relapse free survival
OS	Overall survival
nmCRC	Non-metastatic colorectal cancer
HR	Hazard ratio
CI	Confidence interval
CTLs	Cytotoxic T lymphocytes
MS	Multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
CSF	Cerebrospinal fluid
IFN-γ	Interferon γ
ASCs	Antibody-secreting cells
GZMA	Granzyme A
GZMH	Granzyme H
GZMK	Granzyme K
PRF1	Perforin 1
APCs	Antigen-presenting cells
NPSLE	Neuropsychiatric lupus erythematosus
SLE	Systemic lupus erythematosus
mOS	Median overall survival
TTF	Tumour treatment fields
ICI	Immune checkpoint inhibitors
S1P1	Sphingosine-1-phosphate receptor 1
CCL2	Chemokine ligand 2
CCR7	C-C chemokine receptor type 7
CXCR5	C-X-C chemokine receptor type 5
IL-10	Interleukin-10
IL-17	Interleukin-17
IL-7	Interleukin-7
ILC3	Innate lymphoid cells 3
IDO1	Indoleamine 2,3-dioxygenase 1
TGF-β	Transforming growth factor β
BBB	Blood-brain barrier
LECs	Lymphatic endothelial cells
TCGA	The cancer genome atlas
IDH	Isocitrate dehydrogenase
NSCLC	Non-small cell lung cancer
FoxP3	Forkhead box P3
PD-(L)1	Programmed death-ligand 1
AAV	Adeno-associated viral
PAMPs	Pathogen-associated molecular patterns
TLR	Toll-like receptor
PFS	Prolonged progression-free survival
HCMV	Human cytomegalovirus
IE1	Immediate early protein 1
CAR-T	Chimeric antigen receptor T
STING	Stimulator of interferon genes
GSCs	Glioma stem cells
TAAs	Tumour-associated antigens
iTLS	Inducible TLS

FBR	Foreign body reactions
αCD40	Agonistic CD40 antibodies

Acknowledgements
Not applicable.

Author contributions
Conceptualization, Yang Gao, Wenhao Xu; Investigation and Writing Original Draft, Yang Gao, Wenhao Xu; Writing- Review & Editing, Jiatong Chen, Yuechao Yang; Visualization, Shuang Luan, Yang Gao; Funding Acquisition, Yang Gao; Supervision, Yang Gao, Wenhao Xu.

Funding
This work was supported by National Natural Science Foundation of China (No. 82103429), Noncommunicable Chronic Diseases-National Science and Technology Major Project (No. 2023ZD0510300), National Natural Science Foundation of China (No. 82403377), Beijing Xisike Clinical Oncology Research Foundation (No. Y-Young2024-0138), and China Postdoctoral Science Foundation (No. 2024M750538). FDUROP (Fudan Undergraduate Research Opportunities Program) (No.24012) and National Undergraduate Training Program on Innovation and Entrepreneurship grant (No.202410246111).

Data availability
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors have read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Received: 4 March 2025 / Accepted: 16 April 2025
Published online: 09 May 2025

References

1. De Boeck A, Ahn BY, D'Mello C, Lun X, Menon SV, Alshehri MM, Szulzewsky F, Shen Y, Khan L, Dang NH, et al. Glioma-derived IL-33 orchestrates an inflammatory brain tumor microenvironment that accelerates glioma progression. Nat Commun. 2020;11:4997.
2. Zhao L, Jin S, Wang S, Zhang Z, Wang X, Chen Z, Wang X, Huang S, Zhang D, Wu H. Tertiary lymphoid structures in diseases: immune mechanisms and therapeutic advances. Signal Transduct Target Ther. 2024;9:225.
3. Sun X, Liu W, Sun L, Mo H, Feng Y, Wu X, Li C, Chen C, Li J, Xin Y et al. Maturation and abundance of tertiary lymphoid structures are associated with the efficacy of neoadjuvant chemoimmunotherapy in resectable non-small cell lung cancer. J Immunother Cancer. 2022:10.
4. Wang Q, Shen X, An R, Bai J, Dong J, Cai H, Zhu H, Zhong W, Chen W, Liu A, Du J. Peritumoral tertiary lymphoid structure and tumor stroma percentage predict the prognosis of patients with non-metastatic colorectal cancer. Front Immunol. 2022;13:962056.
5. Hou X, Li X, Han Y, Xu H, Xie Y, Zhou T, Xue T, Qian X, Li J, Wang HC, et al. Triple-negative breast cancer survival prediction using artificial intelligence through integrated analysis of tertiary lymphoid structures and tumor budding. Cancer. 2024;130:1499–512.
6. van de Walle T, Vaccaro A, Ramachandran M, Pietila I, Essand M, Dimberg A. Tertiary lymphoid structures in the central nervous system: implications for glioblastoma. Front Immunol. 2021;12:724739.
7. Xu W, Lu J, Tian X, Ye S, Wei S, Wang J, Anwaier A, Qu Y, Liu W, Chang K, et al. Unveiling the impact of tertiary lymphoid structures on immunotherapeutic responses of clear cell renal cell carcinoma. MedComm (2020). 2024;5:e461.
8. Yang F, Yang J, Wu M, Chen C, Chu X. Tertiary lymphoid structures: new immunotherapy biomarker. Front Immunol. 2024;15:1394505.
9. Langouo FM, Padonou F, Willard-Gallo K. Tumor-associated tertiary lymphoid structures in cancer: implications for immunotherapy. Expert Rev Clin Immunol. 2024;20:839–47.
10. Schumacher TN, Thommen DS. Tertiary lymphoid structures in cancer. Science. 2022;375:eabf9419.
11. Rodriguez AB, Peske JD, Woods AN, Leick KM, Mauldin IS, Meneveau MO, Young SJ, Lindsay RS, Melssen MM, Cyranowski S, et al. Immune mechanisms orchestrate tertiary lymphoid structures in tumors via cancer-associated fibroblasts. Cell Rep. 2021;36:109422.
12. You X, Koop K, Weigert A. Heterogeneity of tertiary lymphoid structures in cancer. Front Immunol. 2023;14:1286850.
13. Cupedo T, Mebius RE. Role of chemokines in the development of secondary and tertiary lymphoid tissues. Semin Immunol. 2003;15:243–8.
14. Borelli A, Irla M. Lymphotoxin: from the physiology to the regeneration of the thymic function. Cell Death Differ. 2021;28:2305–14.
15. Bovay E, Sabine A, Prat-Luri B, Kim S, Son K, Willrodt AH, Olsson C, Halin C, Kiefer F, Betsholtz C, et al. Multiple roles of lymphatic vessels in peripheral lymph node development. J Exp Med. 2018;215:2760–77.
16. Zhang N, Liu X, Qin J, Sun Y, Xiong H, Lin B, Liu K, Tan B, Zhang C, Huang C, et al. LIGHT/TNFSF14 promotes CAR-T cell trafficking and cytotoxicity through reversing immunosuppressive tumor microenvironment. Mol Ther. 2023;31:2575–90.
17. Ramachandran M, Vaccaro A, van de Walle T, Georganaki M, Lugano R, Vemuri K, Kouroughkiaouri D, Vazaios K, Hedlund M, Tsaridou G, et al. Tailoring vascular phenotype through AAV therapy promotes anti-tumor immunity in glioma. Cancer Cell. 2023;41:1134–51.
18. Guedj K, Abitbol Y, Cazals-Hatem D, Morvan M, Maggiori L, Panis Y, Bouhnik Y, Caligiuri G, Corcos O, Nicoletti A. Adipocytes orchestrate the formation of tertiary lymphoid organs in the creeping fat of Crohn's disease affected mesentery. J Autoimmun. 2019;103:102281.
19. Peske JD, Thompson ED, Gemta L, Baylis RA, Fu YX, Engelhard VH. Effector lymphocyte-induced lymph node-like vasculature enables Naive T-cell entry into tumours and enhanced anti-tumour immunity. Nat Commun. 2015;6:7114.
20. Guedj K, Khallou-Laschet J, Clement M, Morvan M, Gaston AT, Fornasa G, Dai J, Gervais-Taurel M, Eberl G, Michel JB, et al. M1 macrophages act as LTbetaR-independent lymphoid tissue inducer cells during atherosclerosis-related lymphoid neogenesis. Cardiovasc Res. 2014;101:434–43.
21. Lochner M, Ohnmacht C, Presley L, Bruhns P, Si-Tahar M, Sawa S, Eberl G. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORgamma t and LTi cells. J Exp Med. 2011;208:125–34.
22. Carrega P, Loiacono F, Di Carlo E, Scaramuccia A, Mora M, Conte R, Benelli R, Spaggiari GM, Cantoni C, Campana S, et al. NCR(+)ILC3 concentrate in human lung cancer and associate with intratumoral lymphoid structures. Nat Commun. 2015;6:8280.
23. Peters A, Pitcher LA, Sullivan JM, Mitsdoerffer M, Acton SE, Franz B, Wucherpfennig K, Turley S, Carroll MC, Sobel RA, et al. Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. Immunity. 2011;35:986–96.
24. Randall TD. Bronchus-associated lymphoid tissue (BALT) structure and function. Adv Immunol. 2010;107:187–241.
25. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019;19:307–25.
26. Sharma S, Kadam P, Dubinett S. CCL21 programs immune activity in tumor microenvironment. Adv Exp Med Biol. 2020;1231:67–78.
27. Wu SY, Zhang SW, Ma D, Xiao Y, Liu Y, Chen L, Song XQ, Ma XY, Xu Y, Chai WJ, et al. CCL19(+) dendritic cells potentiate clinical benefit of anti-PD-(L)1 immunotherapy in triple-negative breast cancer. Med (N Y). 2023;4:373–93.
28. Flanagan K, Moroziewicz D, Kwak H, Horig H, Kaufman HL. The lymphoid chemokine CCL21 costimulates Naive T cell expansion and Th1 polarization of non-regulatory CD4 + T cells. Cell Immunol. 2004;231:75–84.
29. Rouanne M, Arpaia N, Marabelle A. CXCL13 shapes tertiary lymphoid structures and promotes response to immunotherapy in bladder cancer. Eur J Cancer. 2021;151:245–8.
30. Yang M, Lu J, Zhang G, Wang Y, He M, Xu Q, Xu C, Liu H. CXCL13 shapes immunoactive tumor microenvironment and enhances the efficacy of PD-1 checkpoint Blockade in high-grade serous ovarian cancer. J Immunother Cancer. 2019:9.
31. Dai S, Zeng H, Liu Z, Jin K, Jiang W, Wang Z, Lin Z, Xiong Y, Wang J, Chang Y et al. Intratumoral CXCL13(+)CD8(+)T cell infiltration determines poor clinical

outcomes and immunoevasive contexture in patients with clear cell renal cell carcinoma. J Immunother Cancer 2021, 9.
32. Xu W, Ma C, Liu W, Anwaier A, Tian X, Shi G, Qu Y, Wei S, Zhang H, Ye D. Prognostic value, DNA variation and Immunologic features of a tertiary lymphoid structure-related chemokine signature in clear cell renal cell carcinoma. Cancer Immunol Immunother. 2022;71:1923–35.
33. He W, Zhang D, Liu H, Chen T, Xie J, Peng L, Zheng X, Xu B, Li Q, Jiang J. The high level of tertiary lymphoid structure is correlated with superior survival in patients with advanced gastric Cancer. Front Oncol. 2020;10:980.
34. Silina K, Soltermann A, Attar FM, Casanova R, Uckeley ZM, Thut H, Wandres M, Isajevs S, Cheng P, Curioni-Fontecedro A, et al. Germinal centers determine the prognostic relevance of tertiary lymphoid structures and are impaired by corticosteroids in lung squamous cell carcinoma. Cancer Res. 2018;78:1308–20.
35. Posch F, Silina K, Leibl S, Mundlein A, Moch H, Siebenhuner A, Samaras P, Riedl J, Stotz M, Szkandera J, et al. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. Oncoimmunology. 2018;7:e1378844.
36. Rajamanickam JGA, Bui V, Bernard C, Pucilowska B, Ballesteros-Merino J, Schmidt C, McCarty M, Philips K, Piening M. Germinal center reactions in tertiary lymphoid structures associate with neoantigen burden, humoral immunity and long-term survivorship in pancreatic cancer. Oncoimmunology. 2021;10:1900635.
37. Xu W, Lu J, Liu WR, Anwaier A, Wu Y, Tian X, Su JQ, Qu YY, Yang J, Zhang H, Ye D. Heterogeneity in tertiary lymphoid structures predicts distinct prognosis and immune microenvironment characterizations of clear cell renal cell carcinoma. J Immunother Cancer 2023, 11.
38. Ding GY, Ma JQ, Yun JP, Chen X, Ling Y, Zhang S, Shi JY, Chang YQ, Ji Y, Wang XY, et al. Distribution and density of tertiary lymphoid structures predict clinical outcome in intrahepatic cholangiocarcinoma. J Hepatol. 2022;76:608–18.
39. Shang T, Jiang T, Lu T, Wang H, Cui X, Pan Y, Xu M, Pei M, Ding Z, Feng X, et al. Tertiary lymphoid structures predict the prognosis and immunotherapy response of cholangiocarcinoma. Front Immunol. 2023;14:1166497.
40. Meylan M, Petitprez F, Lacroix L, Di Tommaso L, Roncalli M, Bougoüin A, Laurent A, Amaddeo G, Sommacale D, Regnault H, et al. Early hepatic lesions display immature tertiary lymphoid structures and show elevated expression of immune inhibitory and immunosuppressive molecules. Clin Cancer Res. 2020;26:4381–9.
41. Wakasu S, Tagawa T, Haratake N, Kinoshita F, Oku Y, Ono Y, Takenaka T, Oda Y, Shimokawa M, Mori M. Preventive effect of tertiary lymphoid structures on lymph node metastasis of lung adenocarcinoma. Cancer Immunol Immunother. 2023;72:1823–34.
42. Pikor NB, Astarita JL, Summers-Deluca L, Galicia G, Qu J, Ward LA, Armstrong S, Dominguez CX, Malhotra D, Heiden B, et al. Integration of Th17- and Lymphotoxin-Derived signals initiates Meningeal-Resident stromal cell remodeling to propagate neuroinflammation. Immunity. 2015;43:1160–73.
43. Morille J, Mandon M, Rodriguez S, Roulois D, Leonard S, Garcia A, Wiertlewski S, Le Page E, Berthelot L, Nicot A et al. Multiple sclerosis CSF is enriched with follicular T cells displaying a Th1/Eomes signature. Neurol Neuroimmunol Neuroinflamm 2022, 9.
44. Herich S, Schneider-Hohendorf T, Rohlmann A, Khaleghi GM, Schulte-Mecklenbeck A, Zondler L, Janoschka C, Ostkamp P, Richter J, Breuer J, et al. Human CCR5high effector memory cells perform CNS parenchymal immune surveillance via GZMK-mediated transendothelial diapedesis. Brain. 2019;142:3411–27.
45. Epps SJ, Coplin N, Luthert PJ, Dick AD, Coupland SE, Nicholson LB. Features of ectopic lymphoid-like structures in human uveitis. Exp Eye Res. 2020;191:107901.
46. Heng JS, Hackett SF, Stein-O'Brien GL, Winer BL, Williams J, Goff LA, Nathans J. Comprehensive analysis of a mouse model of spontaneous uveoretinitis using single-cell RNA sequencing. Proc Natl Acad Sci U S A. 2019;116:26734–44.
47. Kielczewski JL, Horai R, Jittayasothorn Y, Chan CC, Caspi RR. Tertiary lymphoid tissue forms in retinas of mice with spontaneous autoimmune uveitis and has consequences on visual function. J Immunol. 2016;196:1013–25.
48. Stock AD, Der E, Gelb S, Huang M, Weidenheim K, Ben-Zvi A, Putterman C. Tertiary lymphoid structures in the choroid plexus in neuropsychiatric lupus. JCI Insight 2019, 4.
49. Gelb S, Stock AD, Anzi S, Putterman C, Ben-Zvi A. Mechanisms of neuropsychiatric lupus: the relative roles of the blood-cerebrospinal fluid barrier versus blood-brain barrier. J Autoimmun. 2018;91:34–44.
50. van Solinge TS, Nieland L, Chiocca EA, Broekman M. Advances in local therapy for glioblastoma - taking the fight to the tumour. Nat Rev Neurol. 2022;18:221–36.
51. Jiang T, Nam DH, Ram Z, Poon WS, Wang J, Boldbaatar D, Mao Y, Ma W, Mao Q, You Y, et al. Clinical practice guidelines for the management of adult diffuse gliomas. Cancer Lett. 2021;499:60–72.
52. Khasraw M, Reardon DA, Weller M, Sampson JH. PD-1 inhibitors: do they have a future in the treatment of glioblastoma?? Clin Cancer Res. 2020;26:5287–96.
53. Cloughesy TF, Mochizuki AY, Orpilla JR, Hugo W, Lee AH, Davidson TB, Wang AC, Ellingson BM, Rytlewski JA, Sanders CM, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med. 2019;25:477–86.
54. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541:321–30.
55. Khan F, Pang L, Dunterman M, Lesniak MS, Heimberger AB, Chen P. Macrophages and microglia in glioblastoma: heterogeneity, plasticity, and therapy. J Clin Invest 2023, 133.
56. Chongsathidkiet P, Jackson C, Koyama S, Loebel F, Cui X, Farber SH, Woroniecka K, Elsamadicy AA, Dechant CA, Kemeny HR, et al. Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. Nat Med. 2018;24:1459–68.
57. Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. Curr Opin Immunol. 2015;33:55–63.
58. Wei J, Chen P, Gupta P, Ott M, Zamler D, Kassab C, Bhat KP, Curran MA, de Groot JF, Heimberger AB. Immune biology of glioma-associated macrophages and microglia: functional and therapeutic implications. Neuro Oncol. 2020;22:180–94.
59. Pang L, Khan F, Heimberger AB, Chen P. Mechanism and therapeutic potential of tumor-immune symbiosis in glioblastoma. Trends Cancer. 2022;8:839–54.
60. Liu Y, Zhou F, Ali H, Lathia JD, Chen P. Immunotherapy for glioblastoma: current State, challenges, and future perspectives. Cell Mol Immunol. 2024;21:1354–75.
61. Mo F, Pellerino A, Soffietti R, Ruda R. Blood-Brain barrier in brain tumors: biology and clinical relevance. Int J Mol Sci 2021, 22.
62. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, et al. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015;523:337–41.
63. Eide PK, Vatnehol S, Emblem KE, Ringstad G. Magnetic resonance imaging provides evidence of glymphatic drainage from human brain to cervical lymph nodes. Sci Rep. 2018;8:7194.
64. Rua R, McGavern DB. Advances in meningeal immunity. Trends Mol Med. 2018;24:542–59.
65. Milutinovic S, Abe J, Godkin A, Stein JV, Gallimore A. The dual role of high endothelial venules in Cancer progression versus immunity. Trends Cancer. 2021;7:214–25.
66. Hannocks MJ, Pizzo ME, Huppert J, Deshpande T, Abbott NJ, Thorne RG, Sorokin L. Molecular characterization of perivascular drainage pathways in the murine brain. J Cereb Blood Flow Metab. 2018;38:669–86.
67. Pikor NB, Cupovic J, Onder L, Gommerman JL, Ludewig B. Stromal cell niches in the inflamed central nervous system. J Immunol. 2017;198:1775–81.
68. Zhou X, Li W, Yang J, Qi X, Chen Y, Yang H, Chu L. Tertiary lymphoid structure stratifies glioma into three distinct tumor subtypes. Aging. 2021;13:26063–94.
69. Bastiancich C, Danhier P, Préat V, Danhier F. Anticancer drug-loaded hydrogels as drug delivery systems for the local treatment of glioblastoma. J Control Release. 2016;243:29–42.
70. Franiak-Pietryga I, Miyauchi S, Kim SS, Sanders PD, Sumner W, Zhang L, Mundt AJ, Califano JA, Sharabi AB. Activated B cells and plasma cells are resistant to radiation therapy. Int J Radiat Oncol Biol Phys. 2022;112:514–28.
71. Cai W, Jing M, Gu Y, Bei T, Zhao X, Chen S, Wen J, Gao J, Wu C, Xue Z. Tumor microenvironment features Decipher the outperformance of neoadjuvant immunochemotherapy over chemotherapy in resectable non-small cell lung cancer. Front Immunol. 2022;13:984666.
72. Vanhersecke L, Brunet M, Guégan JP, Rey C, Bougouin A, Cousin S, Moulec SL, Besse B, Loriot Y, Larroquette M, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. Nat Cancer. 2021;2:794–802.
73. Dongye Z, Li J, Wu Y. Toll-like receptor 9 agonists and combination therapies: strategies to modulate the tumour immune microenvironment for systemic anti-tumour immunity. Br J Cancer. 2022;127:1584–94.
74. Shen S, Cui Y, Li M, Yu K, Zhu Q, Zhang X, Shen W, Li H, Jiang H, Li M et al. TLR agonists promote formation of tertiary lymphoid structure and improve anti-glioma immunity. Neuro Oncol 2024.

75. Wen L, Wang XZ, Qiu Y, Zhou YP, Zhang QY, Cheng S, Sun JY, Jiang XJ, Rayner S, Britt WJ, et al. SOX2 downregulation of PML increases HCMV gene expression and growth of glioma cells. PLoS Pathog. 2023;19:e1011316.
76. Yang X, Jiang S, Liu F, Li Z, Liu W, Zhang X, Nan F, Li J, Yu M, Wang Y, Wang B. HCMV IE1/IE1mut therapeutic vaccine induces tumor regression via intratumoral tertiary lymphoid structure formation and peripheral immunity activation in glioblastoma multiforme. Mol Neurobiol. 2024;61:5935–49.
77. Sabahi M, Salehipour A, Bazl M, Rezaei N, Mansouri A, Borghei-Razavi H. Local immunotherapy of glioblastoma: A comprehensive review of the concept. J Neuroimmunol. 2023;381:578146.
78. Qu C, Zhang H, Cao H, Tang L, Mo H, Liu F, Zhang L, Yi Z, Long L, Yan L, et al. Tumor buster - where will the CAR-T cell therapy 'missile' go? Mol Cancer. 2022;21:201.
79. Zhang H, Xu X, Li S, Huang H, Zhang K, Li W, Wang X, Yang J, Yin X, Qu C, et al. Advances in nanoplatform-based multimodal combination therapy activating STING pathway for enhanced anti-tumor immunotherapy. Colloids Surf B Biointerfaces. 2025;250:114573.
80. Emir SM, Karaoğlan BS, Kaşmer R, Şirin HB, Sarıyıldız B, Karakaş N. Hunting glioblastoma recurrence: glioma stem cells as retrospective targets. Am J Physiol Cell Physiol. 2025;328:C1045–61.
81. Aoyama S, Nakagawa R, Mulé JJ, Mailloux AW. Inducible tertiary lymphoid structures: promise and challenges for translating a new class of immunotherapy. Front Immunol. 2021;12:675538.
82. Pollack KE, Meneveau MO, Melssen MM, Lynch KT, Koeppel AF, Young SJ, Turner S, Kumar P, Sol-Church K, Mauldin IS, Slingluff CJ. Incomplete Freund's adjuvant reduces arginase and enhances Th1 dominance, TLR signaling and CD40 ligand expression in the vaccine site microenvironment. J Immunother Cancer 2020, 8.
83. Sadeghirad H, Monkman J, Tan CW, Liu N, Yunis J, Donovan ML, Moradi A, Jhaveri N, Perry C, Adams MN, et al. Spatial dynamics of tertiary lymphoid aggregates in head and neck cancer: insights into immunotherapy response. J Transl Med. 2024;22:677.
84. Gorvel L, Panouillot M, Rouvière MS, Billon E, Fattori S, Sonongbua J, Boucherit N, Ben AA, Quilichini O, Granjeaud S et al. Tertiary lymphoid structures are associated with enhanced macrophage activation and immune checkpoint expression, and predict outcome in cervical cancer. Cancer Immunol Res 2025.
85. Li DK, Wang W. Characteristics and clinical trial results of agonistic anti-CD40 antibodies in the treatment of malignancies. Oncol Lett. 2020;20:176.
86. van Hooren L, Vaccaro A, Ramachandran M, Vazaios K, Libard S, van de Walle T, Georganaki M, Huang H, Pietila I, Lau J, et al. Agonistic CD40 therapy induces tertiary lymphoid structures but impairs responses to checkpoint Blockade in glioma. Nat Commun. 2021;12:4127.
87. Lee-Chang C, Miska J, Hou D, Rashidi A, Zhang P, Burga RA, Jusué-Torres I, Xiao T, Arrieta VA, Zhang DY et al. Activation of 4-1BBL + B cells with CD40 agonism and IFNγ elicits potent immunity against glioblastoma. J Exp Med 2021, 218.
88. Pikor NB, Prat A, Bar-Or A, Gommerman JL. Meningeal tertiary lymphoid tissues and multiple sclerosis: A gathering place for diverse types of immune cells during CNS autoimmunity. Front Immunol. 2015;6:657.
89. Iorgulescu JB, Gokhale PC, Speranza MC, Eschle BK, Poitras MJ, Wilkens MK, Soroko KM, Chhoeu C, Knott A, Gao Y, et al. Concurrent dexamethasone limits the clinical benefit of immune checkpoint Blockade in glioblastoma. Clin Cancer Res. 2021;27:276–87.

Publisher's note