INNOVATIONS JOURNALS

Review Article

The Role of Interleukin-7 in the Formation of Tertiary Lymphoid Structures and Their Prognostic Value in Gastrointestinal Cancers

Michael Brandon Ware,[1] Alexandra A. Wolfarth,[1] Jack B. Goon,[1] Ugonna I. Ezeanya,[1] Swati Dhar,[1] Sara Ferrando-Martinez,[1] Byung Ha Lee[1]

[1]NeoImmuneTech, Inc., Rockville, MD, USA

Address correspondence to Byung Ha Lee (Blee@neoimmunetech.com).

Source of Support: This work was completed as part of employment for NeoImmuneTech, Inc. Conflict of Interest: None.

Received: May 19, 2022; Revision Received: Aug 19, 2022; Accepted: Aug 25, 2022

Ware MB, Wolfarth AA, Goon JB, et al. The Role of interleukin-7 in the formation of tertiary lymphoid structures and their prognostic value in gastrointestinal cancers. *J Immunother Precis Oncol*. 2022; 5:105–117. DOI: 10.36401/JIPO-22-10.

ABSTRACT

Immunotherapies for the treatment of solid tumors continue to develop in preclinical and clinical research settings. Unfortunately, for many patients the tumor fails to respond or becomes resistant to therapies such as checkpoint inhibitors (CPIs) targeting programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen-4 (CTLA-4). In many cancers, failed response to CPIs can be attributed to poor T cell infiltration, dominant immunosuppression, and exhausted immune responses. In gastrointestinal (GI) cancers T cell infiltration can be dismal, with several reports finding that $CD8^+$ T cells compose less than 2% of all cells within the tumor. Organized aggregates of lymphocytes, antigen-presenting cells, and vessels, together termed tertiary lymphoid structures (TLSs), are hypothesized to be a major source of T cells within solid tumors. The intratumoral formation of these organized immune centers appears to rely on intricate cytokine and chemokine signaling to heterogeneous cell populations such as B and T cells, innate lymphoid cells, fibroblasts, and dendritic cells. In GI cancers, the presence and density of TLSs provide prognostic value for predicting outcome and survival. Further, TLS presence and density associates with favorable responses to CPIs in many cancers. This review highlights the prognostic value of TLSs in GI cancers, the role of the homeostatic cytokine interleukin-7 (IL-7) in TLS formation, and the induction of TLSs in solid tumors by novel therapeutics.

Keywords: interleukin-7, tertiary lymphoid structures, gastrointestinal cancer, immunotherapy, cytokines

INTRODUCTION

Impactful treatment of solid tumors with immunotherapy actively fuels research in tumor immunology and patient care. Despite some success, many tumors fail to respond to immunotherapies or the disease progresses and relapses after transient immune responses.[1–3] The fundamental question surrounding differing outcomes in response to immunotherapies, such as checkpoint inhibitors (CPIs), is what underlying biology separates responders and nonresponders. Mechanisms mediating infiltration, expansion, and cytolytic activity of T cells within patient tumors are of utmost interest. Technologic advances in tissue analysis by microscopy and immunofluorescent imaging have provided valuable information on spatial dynamics of immunologic responses within tumors, potentially shedding light on causes for differing responses to immunotherapy.[4–6] These techniques have recently paved the way for the discovery and observation of tertiary lymphoid structures (TLSs) within solid tumors.[4,6]

Like secondary lymphoid organs (SLOs), TLSs contain B cell and T cell zones nurtured by the presence of antigen-presenting cells (APCs) and fibroblasts.[5,6] Architecture such as vasculature and specialized matrices are also similar between SLOs and TLSs, supporting the coordinated influx of naïve cells and efflux of antigen-primed effector cells.[7–10] Whereas SLOs are both encapsulated and established during host development, TLSs are not encapsulated and form in situ as organized lymphoid aggregates responding to distinct inflammatory signals such as viral infection, vaccination, autoimmunity, and cancer.[11–13] TLSs are hypothesized to be a major source of intratumoral T cells in solid tumors, presumably driving potent antitumor responses and acting as a center for lymphocyte activation, proliferation, and maturation.[4] Mechanisms mediating the formation of TLSs within solid tumors continue to be

elucidated, as do their correlation with adaptive immune responses to cancer. Clinical evidence demonstrates TLS presence and density positively correlate with response to CPIs,[14,15] raising the possibility that manipulation of TLS formation in patients could prime responses to immune-based therapies.

Given the immunologically "cold" nature of gastrointestinal (GI) cancers,[16] strategies to mount and stimulate immune responses to these diseases are desperately needed. Single-agent treatment of GI cancers with CPIs have been largely unsuccessful, as have attempts to increase T cell infiltration.[17–23] Increased density or percent area, or simply the presence of TLSs in GI cancers, such as pancreatic ductal adenocarcinoma (PDAC),[24,25] gastric cancer ,[10,15,26–28] hepatocellular carcinoma (HCC), and biliary tract cancer (BTC),[29] positively correlate with better outcomes. One potential strategy for combination treatments with CPIs is to bolster T cell infiltration through increased TLS formation, thereby priming responses to CPIs in GI cancers. Achieving this outcome will require extensive knowledge of initial steps driving TLS formation and intensive investigation of TLS characteristics and functionality in GI cancers. Soluble factors such as cytokines and chemokines coordinate intricate cellular dynamics in TLS formation and are indispensable for successful TLS establishment and maturation. As described later in this review, the homeostatic cytokine interleukin-7 (IL-7) is of particular interest given its central role in TLS formation, and its ability to modulate and expand heterogeneous cell populations supporting TLS formation and maturation. Specifically, IL-7 expands naïve and memory T cells in preclinical and clinical studies and demonstrates promising effectiveness against cancers in combination with CPIs.[30–32] Here, we review current clinical data concerning the prognostic value and characteristics of TLSs in multiple GI cancers, highlighting the similarities and differences in these heterogeneous diseases. We also discuss the role of IL-7, among other soluble factors, in TLS formation and maturation to highlight potential leverage points for therapeutic induction of TLS in patients. Finally, we evaluate cytokine- and chemokine-based immune therapies, which have demonstrated the capability to induce intratumoral TLS in clinical or preclinical settings.

DETECTION AND QUANTIFICATION OF TERTIARY LYMPHOID STRUCTURES WITHIN PATIENT TUMORS

The interpretation of TLSs as a prognostic or predictive biomarker is complicated by the lack of universal methods and assays for the quantification and assessment of TLS in patient tissue. Multispectral immunofluorescence or immunohistochemistry is used to quantify B cell and T cell zones in TLSs using the markers CD3, CD4, CD8, and CD20.[25,29,33–36] Follicular dendritic cells (FDCs) are also stained for using antibodies to CD21 and/or CD23[25,35]; CD208 has also been used to detect dendritic cells (DCs).[33] High endothelial venules (HEVs), which are key for lymphocyte movement into TLSs, are identified by positive staining for peripheral node addressin.[10,34] The above markers are sufficient to separate lymphoid aggregates from true, organized TLS. Unfortunately, not all clinical settings are equipped to incorporate these technically complex analyses. Thus, a few reports solely use H&E-stained [26,37] or CD20-stained[15] sections to quantify TLSs in patient tumors; however, these approaches do not allow for rigorous characterization of TLSs over less organized lymphoid aggregates. Further adding to discordance between studies, some groups use serial sections to ensure detection of cell types throughout the depth of the tissue[33] or to pair immunohistochemistry with H&E staining.[35] Others use a single section and therefore inadvertently limit the scope of sampling within the tumor lesion. The quantification of TLS in resulting images provides yet another obstacle in terms of study-to-study concordance. Most frequently, TLS are quantified with the help of a pathologist as density (TLS/mm^2 of tissue)[26,29,35]; however, percent area[15] or simply absence vs presence[33] has also been used in clinical studies to quantify TLSs. The size and composition of TLSs have proven to be a useful measure in some cases, but these aspects of TLSs are not consistently considered. Going forward, efforts should be made within the field to arrive at a consensus for tissue sampling and measurement of TLSs in patient tissue with room for more extensive characterization to be incorporated.

TERTIARY LYMPHOID STRUCTURES IN GASTROINTESTINAL MALIGNANCIES PREDICT SURVIVAL AND ASSOCIATE WITH DISTINCT IMMUNE RESPONSES

GI malignancies with genomic instabilities have increased immunogenicity, high levels of T cell infiltration, and favorable response to immune-based therapies.[38–40] However, genomically stable (GS) or microsatellite stable (MSS) tumors are notoriously resistant to single-agent CPIs owing to low immunogenicity, poor T-cell infiltration, and broad immunosuppression.[38–40] Prognostic indicators of response to CPIs in patients with GS tumors are desperately needed as combinatorial treatment strategies are developed to target these resistant tumors. Most recently, immunohistochemical examination of GI tumors has demonstrated TLSs to associate with increased T cell and B cell infiltration, APCs, and distinct immune signatures (Table 1). Although a limited number of studies have explored the relationship between TLSs and response to CPIs in GI cancer, early results indicate a positive association between the two. Indeed, a growing number of studies have demonstrated direct association between increased TLS density, percent area, or presence in GI cancers and better clinical outcome for patients (Table 2). A deeper

Table 1. TLS associate with distinct immunologic signatures in gastrointestinal tumor specimens

Author, Year	TLS-Associated Features
Gastric Cancer	
Schlosser et al, 2019[10]	TLS physically associated with differentiated and organized B-cell infiltrates. Follicular T helper cells were enriched in TLS-positive tumors.
Yamakoshi et al, 2020[27]	TLS were identified by clustering of CD20+ cells and measured as density of TLS per mm^2 of tissue. TLS positively associated with TIBs.
Sakimura et al, 2017[28]	TLS contained CD21+ follicular dendritic cells and Bcl6+ B cells. CD20+ B cells were significantly associated with intratumoral and peritumoral CD8+ T cells.
He et al, 2020[26]	TLS density (referred to as TLS-SUM) positively correlated with tumor size, grade, pTN stage, and WHO subtype.
Derks et al, 2020[33]	The presence of TLS was positively associated with better T and B cell function scores.
Mori et al, 2022[15]	TLS^{hi} status, as defined by the authors, positively associated with immune-related adverse events.
Colorectal Cancer	
Zhao et al, 2021[53]	High TLS expression positively associated with smaller tumor size and greater tumor infiltrating T cells.
Pancreatic Cancer	
Kuwabara et al, 2019[59]	TLS density positively associated with higher infiltration of CD20+ B cells into the TLS and higher proportion of HEV within the TLS.
Hiraoka et al, 2015[24]	TLS presence positively associated with more T and B cell infiltration and less Treg and M2 macrophage infiltration. TLS presence also positively correlated with an inflammatory signature of chemokines and cytokines. TLS presence associated with arterioles, venules, and nerves with vascular networks.
Gunderson et al, 2021[25]	Presence of Early-TLS positively associated with CD8 T cells and CD20 mature B cells. Presence of Early-TLS was not associated with CD4 T cells. Presence of TLS-containing germinal center–like B cells is associated with increased frequency of TLS and CD8 T cell infiltration.
Primary Liver Cancer	
Ding et al, 2022[29]	iTLS showed increased Tfh and Treg levels as compared to peritumoral TLS.
Li et al, 2021[35]	High pTLS density was associated with increased expression of Th1, Th17, and immune suppression-related genes, higher infiltration of CD3+, CD8+, and CD20+ cells, and lower infiltration of FOXP3+, CD68+, and PD1+ cells.

HEV: high endothelial venule; iTLS: intratumoral TLS; pTLS: peritumoral TLS; pTN: pathological tumor and lymph node; Tfh, T follicular helper; TIBs: tumor-infiltrating B cells; TILs: tumor-infiltrating lymphocytes; TLS: tertiary lymphoid structures; TLS^{hi}: TLS high frequency; Treg, T regulatory cell; WHO: World Health Organization.

understanding of how TLSs contribute to adaptive immune responses in GI tumors and mediate response to CPIs is needed to successfully develop potent combination treatment strategies for patients with these deadly malignancies.

Tertiary Lymphoid Structures Predict Survival and Response to Therapy in Patients with Gastric Cancer

Gastric cancer arises from the lining of the stomach and most frequently present as adenocarcinoma, as with most GI cancers.[41] Resection-eligible patients are treated with neoadjuvant chemoradiotherapy followed by surgical resection[41]; however, around 60% of patients will experience recurrence after resection, frequently within 2 years of surgery.[42] Patients with genomic-instable or Epstein-Barr virus–positive gastric cancer often present with inflamed tumors that respond favorably to immunotherapy with CPIs.[33,43] Conversely, more than half of patients with gastric cancerpresent with MSS disease with poor T cell infiltration and dismal overall response rates to single-agent programmed cell death protein-1 (PD-1) blockade (pembrolizumab).[33,43,44] Thus, there is a distinct bimodal nature to CPI responses in gastric cancer, which is at least partly dependent on genomic stability, as seen in other GI malignancies.[33,43]

Immunohistochemical staining and evaluation of resected gastric cancer has provided valuable insight into signatures that predict survival and responsiveness to CPIs.[10,15,26,28] Several studies found positive associations between the magnitude of tumor-infiltrating B cells (TIB) and improved clinical outcome for patients with gastric cancer.[27] GS and MSS gastric tumors are reported to be enriched for B cells, as well as $CD4^+$ T cells and macrophages, relative to microsatellite instable (MSI) cancers.[33] Immunohistochemistry and flow cytometric analysis of B cells in gastric cancer found that TIB predominantly associate with TLSs.[10,27,28] Rather than differentiating into antibody-producing plasma cells, TIBs in tumors with high frequencies of TLSs (TLS^{hi}) demonstrate an activated germinal center (GC) phenotype.[10,27] Further, TIB in gastric cancer express antigen-presenting signatures and may act to activate and stimulate the proliferation of T cells within TLSs.[27] In line with these findings, TIB positively associated with $CD8^+$ T cell infiltration in gastric cancer.[28] Of note, T cells within TLSs of gastric cancer express CD103, indicating that tissue resident memory T cells either migrate into TLSs or participate in their formation.[15,45]

Several reports found that an increase in TLSs (measured as percent area of surgically resected specimens) is associated with better outcome for patients with gastric cancer, but few studies have explored the association between TLS frequency and response to CPIs.[10,26,27,33] A single retrospective study in gastric cancer analyzed TLSs in macroscopically resected perito-

Table 2. The prognostic value of TLSs in gastrointestinal tumor specimens

Author, Year	TLS Prognostic Value	Treatment	No. of Patients Included in Analysis
Gastric Cancer			
Yamakoshi et al, 2020[27]	TLS richness (measured by B-cell–rich area) positively associated with better prognosis when NLR was low.	Surgery	226
Sakimura et al, 2017[28]	High numbers of CD20+ B cells, which were aggregated into TLS, were associated with better OS.	Surgery	226
He et al, 2020[26]	TLS density (referred to as TLS-SUM) positively correlates with OS.	Surgery	914
Mori et al, 2022[15]	TLS^{hi} status, defined as percentage area of tissue with CD20+ clusters, associated with PRs and greater disease control.	Nivolumab	19
Rozek et al, 2016[52]	Presence of a prominent CLR was prognostic for survival.	Surgery	1491
Zhao et al, 2021[53]	High TLS expression, defined by various criteria, associated with lower clinical grade, lower N stage, lower relapse rates.	Surgery	6647
Chalabi et al, 2020[55]	TLS density per mm^2 of tissue increased on treatment but were not associated with response in either pMMR or dMMR tumors.	Neoadjuvant immune checkpoint inhibition	35
Pancreatic Cancer			
Kuwabara et al, 2019[59]	iTLS density was prognostic for survival and clinical outcome in patients who received NAC as a preoperative treatment.	Preoperative neoadjuvant chemoradiotherapy or surgery first	47
Hiraoka et al, 2015[24]	Presence of iTLS was predictive of survival.	Surgery	308
Gunderson et al, 2021[25]	Presence of TLS correlated with longer OS and DFS. Patients with mature TLS had significantly longer survival than patients with E-TLS. TLS density did not correlate with OS.	Surgery	63 (TLS) and 30 (M-TLS)
Primary Liver Cancer			
Ding et al, 2022[29]	pTLS score associated with decreased OS; iTLS score associated with increased OS.	Surgery	962
Li et al, 2021[35]	pTLS density, especially alongside the presence of iTLS, associated with increased OS.	Surgery	240
Wen et al, 2021[63]	TLS density was associated with increased OS. The combination of TLS density and NLR better predicts survival.	Surgery	85
Calderaro et al, 2019[37]	iTLS-associated gene expression signature associated with lower risk of early relapse.	Surgery	273
Garnelo et al, 2017[64]	T cell and B cell density, as well as the expression of CD40, predicts survival.	Surgery	103, 112, and 54 respectively
Ho et al, 2021[65]	The absolute number of TLS within the tumor was enriched in responders.	Neoadjuvant cabozatinib and nivolumab	12

CLR: Crohn-like lymphoid reaction; DFS: disease-free survival; dMMR: mismatch repair deficient; E-TLS: early-stage TLS; iTLS: intratumoral TLS; M-TLS: mature TLS; NAC: Neoadjuvant Chemotherapy; NLR: neutrophil-to-lymphocyte ratio; OS, overall survival; pMMR: mismatch repair proficient; PRs: partial responses; pTLSs: peritumoral TLS; TLS: tertiary lymphoid structure; TLS^{hi}: TLS high frequency.

neal metastases from 19 patients who underwent resection, had recurrence, and were then treated with nivolumab.[15] The response rate for this cohort of patients was 15.8%, which mirrors that seen in other clinical trials of CPIs and gastric cancer.[15] The percent area staining positive for TLSs was determined by pathology analysis and cases were then classified to be TLS^{hi} or TLS^{low}. Patients with TLS^{hi} tumors demonstrated more frequent immune-related adverse events, and all patients who had a partial response to nivolumab were classified as TLS^{hi}.[15] Further, the disease control rate for patients classified as TLS^{hi} vs TLS^{low} was 66.7% and 20%, respectively.[15] This striking association indicates a potentially complex dynamic between TLSs and disease progression in gastric cancer. However, more data are needed to accurately evaluate the predictive value of TLSs for response to CPIs in patients with gastric cancer.

Tertiary Lymphoid Structures Unique to Colorectal Cancer Predict Survival

Colorectal cancer (CRC) can be classified as d-MMR/MSI-H (mismatch repair deficient/MSI-high) or p-MMR/MSS (mismatch repair proficient/MSS), with MSI-H tumors having significantly more mutations, increased neoantigen load, and a more proinflammatory tumor microenvironment (TME).[46] Increased CPI efficacy is seen in patients with MSI-H CRC tumors and as a result, pembrolizumab and nivolumab with or without ipilimumab are approved for treatment of these patients.[47] Unfortunately, 85% of patients with CRC have MSS tumors and successful treatment with immunotherapy is rare.[48] The size of TLSs that formed in these tumors was evaluated and scored as having high or low TLS activity, with higher TLS activity reflecting larger TLS formation on average as measured by an established histomorpho-

metric method.[49] Average TLS activity score was found to be higher in MSI-H CRC tumors, although there was much more variability in both TLS activity score and infiltration of $CD8^+$ tumor-infiltrating lymphocytes (TIL) than in MSS tumors.[49] This increased variability could explain why response rates to CPIs remains limited in MSI-H tumors.[50] CRC tumors also have peritumoral lymphoid aggregates referred to as Crohn's-like lymphoid reaction (CLR), which despite its name, has no known relationship with Crohn's disease and can be considered a type of TLS specific to CRC.[51] Multivariate analysis has shown that both TILs and CLR have strong prognostic value for survival of patients with CRC, whereas microsatellite status does not.[52] Further underscoring the prognostic significance of TLSs in CRC, meta-analysis revealed that lower clinical grade, lower N stage, and lower relapse rates were associated with higher TLS formation in patents with CRC.[53]

Although TLSs clearly associate with favorable outcome in CRC,[54] there are limited data demonstrating the value of TLSs in determining response to immunotherapy. In the NICHE trial (ClinicalTrials.gov Identifier: NCT03026140), early-stage CRC patients with MSI-H and MSS tumors were given neoadjuvant ipilimumab and nivolumab.[55] After 6 weeks, tumor biopsy specimens were stained for CD20, and TLSs were enumerated. When separating responders from nonresponders, there was no significant difference in the number of TLSs at baseline.[55] The authors also did not detect a significant increase of TLSs in either responders or nonresponders, although a trend towards increased TLSs in responders was present.[55] The only biomarker they found to be significantly associated with response was the number of $CD8^+$ $PD\text{-}1^+$ TILs.[55] To better understand the relationship between TLSs and immunotherapy, more investigation is needed, including more in-depth characterization of the heterogeneous TLSs and CLRs that are found in CRC tumors.[51,56]

Pancreatic Ductal Adenocarcinomas Contain Few Intratumoral Tertiary Lymphoid Structures

PDAC is an immunologically cold tumor characterized by dense fibrosis, a highly inflamed TME, and poor T cell infiltration. The immunosuppressive TME of PDAC is hypothesized to be one cause for low response rates of PDAC to single-agent CPIs. The small subset of patients with MSI disease are the exception to this generalization (1–2% of patients).[57,58] Despite the immunologically cold nature of PDAC,[1,16,21] several published studies have found TLSs in surgical PDAC specimens, albeit at low frequency.[24,59] Stratification of patient tumors by the ratio of TLS area to tumor area provides prognostic value for survival and clinical outcome of patients with PDAC who are receiving neoadjuvant chemotherapy.[59] Notably, the data exist only for patients with resectable disease, while the characteristics and value of TLSs in advanced PDAC are unexplored. One study proceeded to classify 308 patients on the basis of location of TLSs within resected specimens as either intratumoral or peritumoral (on the tumor margin or adjacent, respectively).[24] In line with poor T cell infiltration previously reported in PDAC, only a minority of samples contained TLSs intratumorally. The presence of TLSs within tumors, regardless of number, was highly predictive of survival and correlated positively with T-cell infiltration but inversely with T regulatory cell (Treg) and M2 macrophage infiltration.[24] This inverse correlation between immunosuppressive cell subsets and TLSs is striking, especially given the immunosuppressive nature of PDAC. Further, increased numbers of TLSs in PDAC also correlate with an inflammatory signature of chemokines and cytokines (*IFNG*, *TBX21*, *IL12B*, and *TNF*).[24] It is not clear whether TLSs shift the balance of immunosuppressive subsets in PDAC or arise within tissue after disruption of immunosuppressive inflammatory signals. The relationship between immune suppression and TLSs should continue to be elucidated moving forward.

In PDACs that contain higher TLS counts per tissue section, TLSs demonstrate distinct phenotypes possibly indicative of differences in priming of the adaptive immune response between patient tumors.[25,59] Interestingly, several reports found no association between the absolute number of TLSs per tissue section and total $CD4^+$ T cell infiltration, but increased frequencies of TLSs significantly correlated with higher infiltration of $CD8^+$ T cells and $CD20^+$ mature B cells within the tumor.[25,59] TLSs containing GC-like B cells are associated with increased frequency of TLSs as well as increased $CD8^+$ T cell infiltration.[25,59] Unfortunately, we found no published studies that quantified TLSs in pre-surgical or postsurgical pancreatic tumor specimens from patients treated with CPIs. As combination treatment strategies are explored in clinical trials for patients with pancreatic cancer, the predictive value of TLSs should be investigated.

Peritumoral and Intratumoral Tertiary Lymphoid Structures Are Inversely Correlated with Outcome in Cholangiocarcinoma and Hepatocellular Carcinoma

Primary liver cancer (PLC) is comprised of two major histologic subtypes, cholangiocarcinoma (CC) or BTC and HCC, with distinct etiologies and immune characteristics.[60] Both malignancies are characterized by intense levels of inflammation, which often lead to immunosuppression and impaired T cell responses[60–62]; however, TLSs have been detected in both diseases and at least a subset of patients demonstrate active immune responses. TLSs are described as either intratumoral (iTLS) or peritumoral (pTLS) on the basis of their location within the tumor bed or in adjacent normal liver tissue, respectively.[29] Interestingly, iTLSs and pTLSs seem to contribute differentially to active immune responses in CC vs HCC. A single retrospective analysis of 962 patients with intrahepatic cholangiocarcinoma (iCC) identified an

inverse correlation between iTLSs and pTLSs with respect to survival.[29] This study developed a T score reflective of increased TLSs in the tumor with decreased numbers of TLSs in the peri-tumoral region, and a P score that reflects a reciprocal localization of TLSs. In iCC, a higher P score was associated with decreased overall survival (OS), while a higher T score positively correlated with longer OS, increased T follicular helper (Tfh) cells, and increased Tregs.[29]

In contrast, a higher density of pTLSs (measured as number of TLSs/mm^2), especially in combination with higher iTLS density, positively associated with greater OS in HCC.[35,63] In a separate study of 273 patients with HCC, iTLS density independently associated with a lower risk of early relapse.[37] Thus, both the density and location of TLSs provide prognostic insights for specific populations of patients with liver-associated tumors. Enriched TLSs, orchestrated-B cell responses, and the presence of plasma cells may also identify responders to immunotherapy in HCC.[64] Post-therapeutic surgical resection and analysis of HCC tissue from patients treated with neoadjuvant cabozantinib and nivolumab identified enrichment of these characteristics (TLS density per mm^2, CD3$^+$ and CD8$^+$ T cell density, and CD20$^+$ B cell density) in responders compared to nonresponders.[65] Owing to lack of baseline tissue analysis, it is unclear whether TLSs and B cell signatures are therapeutically induced or rather prime responses to immunotherapy.[65]

The wide-ranging etiologies of PLCs (e.g.., smoking, viral infection, and parasite-induced inflammation) are associated with unique inflammatory responses in the liver.[60] Distinct immune subsets have also been detected between histologic subtypes of CC.[61] In other diseases, etiologies such as human papilloma virus contribute to immune phenotypes and the strength of TLSs as a biomarker of response.[36] A more complete understanding of how iTLSs and pTLSs fuel immunologic responses in specific patient populations, such as hepatitis C– or hepatitis B–positive HCC or liver fluke–induced CC, is needed. Further, many patients with HCC also present with cirrhosis, which is characterized by fibrosis, desmoplasia, and activated myofibroblasts.[60,66] Given the key role of stromal fibroblasts in TLS formation and function, the degree and anatomic location of cirrhosis and activated fibroblasts with respect to HCC and TLSs could impact the value of pTLSs as a biomarker of survival, progression, or therapeutic response. The heterogeneity of these broadly classified tumors presents a challenge to understanding immune response within these tumors but must certainly be taken into account.

THE ROLE OF INTERLEUKIN-7 IN TERTIARY LYMPHOID STRUCTURE FORMATION

The organizational aspects of TLSs, such as B and T cell zones and lymphatic vasculature, distinguish TLSs from ectopic lymphoid aggregates or clusters of TIL. Mature TLSs are intratumoral sites of B and T cell differentiation, reflective of processes observed in SLO such as the spleen or lymph nodes. Although these characteristics define fully formed and mature TLSs, the mechanisms mediating formation of TLSs are still poorly understood. Understanding TLS formation and the signals that stimulate these processes could pave the way for successful induction of TLSs in a higher proportion of patients. Particular attention is being given to secreted proteins such as IL-7 and CC-chemokine ligand 19 (CCL19), which can prime or induce TLS formation, as described below. These cytokines are heavily involved in the expansion of cells that stimulate TLS formation and the initiation of chemotactic signals that regulate the growth and maturation of lymphoid aggregates into TLSs. Here, we will discuss the ligand-receptor interactions required for initiation of TLS formation, and the subsequent maturation of bona fide TLSs, which support adaptive immunologic responses to solid tumors. Whereas comprehensive reviews of TLS formation can be found elsewhere,[67,68] we will instead highlight the role of IL-7 in TLS formation from inception to functional maturity.

Interleukin-7 Promotes Initiation and Establishment of Tertiary Lymphoid Structures Through Lymphoid Tissue Inducers

Initiation and formation of TLSs requires multifaceted ligand-receptor interactions between innate lymphoid cells (ILC), stromal cells, endothelial cells, and B cells. Among these are CD3$^-$CD4$^{-/+}$CXCR5$^+$IL-7Rα^{hi} ILC known as lymphoid tissue inducer (LTi) cells, which are required for the emergence of SLO.[69–72] These cells co-express high levels of IL-7 receptor alpha (IL-7Rα), also known as CD127, and the C-X-C chemokine receptor CXCR5, which binds to C-X-C chemokine ligand 13 (CXCL13) and is proposed to both complement and overlap IL-7 signaling in TLS initiation.[69,73] LTi cells rapidly expand and accumulate in tissues upon increased in vivo availability of IL-7, which can be secreted by fibroblasts or administered ectopically to the host.[69,71,72] IL-7 primarily acts to support survival of cells expressing IL-7Rα and the common gamma chain (γc); however, IL-7 also promotes the expansion and activation of IL-7Rα$^+$ cells and enhances their responsiveness to chemotactic signals such as CXCR5 (Fig. 1). Coordinated signaling through IL-7Rα and CXCR5 to LTi cells drives their expansion, infiltration, and eventual interaction with stromal cells of mesenchymal origin embedded within inflamed tissue.

LTi cells primarily secrete lymphotoxin alpha-1 beta-2 (Ltα1β2), which exists as a membrane-bound heterotrimer and promotes chemokine secretion, T cell infiltration, and de novo vessel formation in solid tissue in a paracrine manner.[74] Lymphotoxin-dependent signaling between LTi cells and lymphotoxin beta receptor

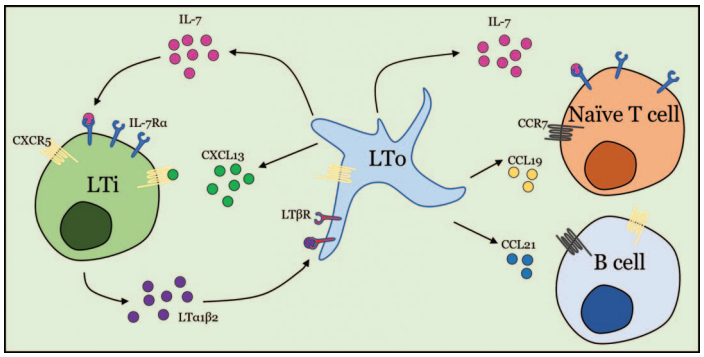

Figure 1. IL-7 and LTα1β2 are part of an integrated cycle. IL-7 induces proliferation and expansion of LTi cells and LTα1β2 secretion by LTi cells. LTα1β2 induces maturation and secretion of CXCL13 and IL-7 by LTo cells. Subsequently, mature LTo cells secrete CCL19 and CCL21 to induce the infiltration of CCR7+ T cells and B cells and support their survival by the secretion of IL-7.

CCL19 and CCL21: CC-chemokine ligands 19 and 21; CCR7: CC chemokine receptor 7; CXCL13: C-X-C chemokine ligand 13; CXCR5: C-X-C chemokine receptor 5; IL-7: interleukin 7; IL-7Rα: IL-7 receptor alpha; LTi: lymphoid tissue inducer; LTo: lymphoid tissue organizer; LTα1β2: lymphotoxin alpha-1 beta-2; LTβR: lymphotoxin beta receptor.

(LTβR)–expressing stromal cells induces secretion of homeostatic chemokines, CXCL13[70], and CC-chemokine ligands 19 and 21 (CCL19 and CCL21).[34,75] LTβR$^+$ lymphoid tissue organizer (LTo) cells of mesenchymal origin possess stromal-organizing capabilities and are primary producers of these chemokines (Fig. 1). In TLSs, LTo cells demonstrate heterogeneous phenotypes and plasticity, with the ability to mature into fibroblasts capable of supporting multiple aspects of TLSs. Even in an immature state, LTo cells can secrete IL-7 and CXCL13 in the absence of Ltα1β2. Subsequently, IL-7 can induce the secretion of Ltα1β2 by LTi cells in a context-dependent manner. Thus, a potential feedforward cycle exists during TLS formation, whereby LTo cells can directly support LTi cell survival and Ltα1β2 secretion in TLSs through IL-7 secretion and, in turn, LTi cells support LTo cell maturation by secreting LTα1β2[72] (Fig. 1).

T Cell Infiltration and Lymphatic Vessel Development in Tertiary Lymphoid Structures Is Governed by the Prosurvival Cytokine Interleukin-7

As stromal cells within TLSs mature and expand, specialized structures such as GCs and HEVs begin to emerge. HEV and lymphatic vessel development in lymphoid organs are the result of lymphatic endothelial cell (LEC) maturation, possibly from cells that originally acted as LTo.[8,9] Although there are distinct differences between LTo cells and LECs, both cells express adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and produce IL-7.[76] Further, LEC-driven lymphangiogenesis in TLSs is reliant on Ltα1β2-secreting cells including LTi cells and infiltrating lymphocytes during early stages of vessel development. Interestingly, both IL-7 and Ltα1β2 can directly signal to LECs to drive the formation of lymphatic vessels and HEVs, possibly through regulation of vascular endothelial growth factor C (VEGF-C).[7] The ability of LECs to secrete IL-7 and respond to IL-7 through IL-7Rα suggests that autocrine signaling through IL-7 could fuel lymphatic vessel development.[76] Given the kinetics of IL-7Rα expression after IL-7 signaling, spatiotemporal IL-7Rα expression by LECs could hypothetically guide the proliferation of LECs and the sprouting of vessels during early stages of TLS development.[76]

LTo cell maturation is accompanied by upregulation of CXCL13, CCL19, and CCL21 to promote B and T cell infiltration into TLSs.[34,77,78] Simultaneously, LTo cells increase their expression and secretion of IL-7 to support the survival and expansion of these lymphocytes. A 12-chemokine signature for detecting early TLS formation was recently published that includes CXCL13, CCL19, and CCL21, among other chemokines,[79] indicating that these chemokines are actively secreted within fully formed TLSs. Together, CXCL13, CCL19, and CCL21 orchestrate the recruitment and distribution of B and T cells to sites of TLS establishment. Mice lacking CXCR5 (the receptor for CXCL13) or CCR7 (the receptor for CCL19 and CCL21) fail to develop peripheral lymph nodes, B cell follicles, Peyer patches, and TLSs, emphasizing the crucial role these chemokine receptors play in lymphoid organ formation and organization.[73] The receptors CXCR5 and CCR7 control the migration and organization of LTi and LTo cells within lymphoid organs, and CXCR5 governs B cell migration within lymphoid organs in a manner similar to B cell follicles.[78,80,81] As TLSs begin expanding and maturing, expression of CXCL13, CCL19, and CCL21 increases and drives the infiltration of CXCR5$^+$ and CCR7$^+$ T and B cells into TLSs through HEVs. The observation that CCR7$^+$ lymphocytes infiltrate tissue and organize within TLSs shifted hypotheses concerning organization of adaptive immunity in cancer, as this marker can identify naïve and stem-like lymphocytes. Rather than T cells becoming primed and maturing into effector or memory cells in tumor-draining lymph nodes, this observation suggests antigen priming of naïve T cells can occur directly within tumors via TLSs. Indeed, innovative studies in preclinical models demonstrate that naïve T cells infiltrate tumors directly, even in the absence of SLO, through lymph node–like vasculature, and differentiate into effector T cells within the tumor.[82–84] Single-cell RNA sequencing of patient tumors has also revealed that a sizeable population of tumor-responsive T cells in patients are not derived from sentinel lymph nodes and may instead be derived from TLSs, further highlighting the potential of TLSs to directly prime tumor-specific T cells in situ.[85]

B and T Cell Differentiation in Tertiary Lymphoid Structures Is Supported by Interleukin-7

Identification of TLSs is dependent on the discernment of distinct B and T cell zones, as with GC in lymphoid organs. This is predicated on the stipulation that TLSs are not just aggregates of lymphocytes but serve as a center for antigen-driven differentiation of naïve lymphocytes into effector and memory subsets. During early stages of TLS development, naïve lymphocytes traffic into TLSs through lymphatic vessels along CXCL13, CCL19, and CCL21 chemokine gradients. As TLSs mature, fewer lymphocytes traffic into these structures; instead, differentiated cytotoxic effector cells traffic out of TLSs into the tumor bed. Innovative studies have revealed that T cell factor 1 (TCF1)–expressing stem-like T cells reside within immune niches such as TLSs and give rise to more differentiated effector T cells.[4] Moreover, the increased infiltration of patient tumors by T cells seemingly depends on this process.[4]

The organization and appearance of mature TLSs harboring stem-like T cells has been compared to B cell follicles.[4] Distinct B and T cell zones and the formation of GCs have also been described in TLSs.[10,25,28,36] Interestingly, GCs require IL-7 for their formation, and increased levels of IL-7 drive GC formation in vivo. In maturity, GC-associated FDCs, along with fibroblastic reticular cells (FRCs) and Tfh cells, support B cell survival, proliferation, and maturation through secretion of survival factors such as IL-7. Further, these supportive cells direct T-cell migration via production of chemokines such as CCL19 and CCL21.[86,87] The presence of GCs within TLSs indicate that TLSs are a site of B cells developing into affinity-matured and class-switched B cells that can recognize antigen and differentiate into memory B cells.[36] B cells with a GC-like signature can distinguish mature and immature TLSs and are associated with better prognosis and outcome in several solid tumors.[25,36]

Although FRCs are primarily thought to support T cell activity, FRCs can also secrete the B cell survival and maturation factors IL-7, BAFF (B-cell-activating factor), and APRIL (a proliferation inducing ligand). Additionally, FRCs in T-cell zones secrete IL-7 to promote T cell survival and proliferation.[88] IL-7 is essential for central memory T cell survival, highlighting the importance of IL-7 in lymphocyte maturation. Fibroblasts in TLSs are thought to mature from LTo cells, driven by LTα1β2 signaling; secrete IL-7 and CXCL13; and express adhesion molecules to support the infiltration, expansion, and differentiation of lymphocytes within TLSs. Although fibroblasts resembling FRCs have been identified in TLSs, cancer-associated fibroblasts demonstrate tremendous heterogeneity and plasticity. Classically, stromal cells are responsible for maintaining homeostatic levels of IL-7 within the periphery and therefore are likely the primary source of IL-7 sustaining T and B cell proliferation, expansion, and differentiation in TLSs. The inherit plasticity, low frequency, and dynamic abilities of FRCs within tumors has made them a challenge to study, and current research seeks to gain a deeper understanding of these cells in cancer. Given the many supportive roles of FRCs in TLSs, manipulation of these fibroblasts represents a potential leverage point for TLS induction by strategic therapeutic intervention.

Altogether, IL-7 plays an essential role in every stage of TLS development, maturation, and functionality. Consequently, as discussed below, different preclinical and clinical approaches are being explored to modulate TLS formation to impact clinical response to immunotherapy.

THERAPEUTIC STRATEGIES TO INDUCE AND ENRICH TERTIARY LYMPHOID STRUCTURE FORMATION WITHIN SOLID TUMORS

Several studies have now demonstrated strong positive correlations between the presence of TLSs in solid tumors and clinical outcome for patients.[24–26,29,35,67] Further, an increasing body of evidence point to TLSs as key mediators of response to immunotherapy.[5,14,15,67,89,90] The induction of TLSs has been associated with inflammatory reactions such as vaccination, viral infection, arthritis, Sjogren's syndrome, and insulitis.[13,91,92] Current strategies to induce TLS formation aim to kick-start antitumor inflammatory responses with strong B and T cell involvement. These strategies include modulation of cytokines and chemokines, infusion of cellular therapies, and administration of vaccine-based therapeutics.

Recombinant Interleukin-7 Therapies Aim to Induce Tertiary Lymphoid Structures and Synergize with Checkpoint Inhibitors

Given the importance of soluble factors in mediating many early events during TLS formation, modulation of these soluble factors by direct administration has emerged as a potential TLS-inducing strategy. Of the chemokines and cytokines involved in TLS formation (CCL19, CCL21, LTα, and IL-7), IL-7–based therapies have advanced most prominently into clinical trials for the treatment of cancer. Administration of IL-7 into the host shows promise as a potential TLS-inducing therapy, with the ability to modulate multiple cell types.[93] IL-7 treatment could initiate many of the signaling cascades described above, driving coordinated signaling by fibroblasts, DCs, and Tfh cells, while simultaneously expanding naïve T cells in the periphery with the ability to traffic into emerging TLSs. Novel strategies are being used to deliver IL-7 to the host or directly into solid tumors, including IL-7– and IL-12–producing oncolytic viruses, IL-7–producing cellular therapies, direct injection of IL-7 into the tumor, or administration of IL-7 systemically. In rhesus macaques, a glycosylated form of IL-7 (R-sIL-7-gly) induced rapid increases in peripheral T

cells expressing CXCR4, CCR6, and CCR7 followed by massive infiltration of T cells into lymph nodes, the skin, and the gut.[94] Interestingly, R-sIL-7-gly also induced upregulation of CCL19 and CCL21 within SLO and the intestines.[94] In addition, administration of a long-lasting Fc-fused mouse IL-7 induced GC formation, expanding Tfh cells and GC B cells in mice.[93]

In the clinical setting, CYT107, a recombinant-human IL-7 developed by RevImmune, has shown the ability to increase circulating $CD4^+$ and $CD8^+$ naïve T cells in patients with solid tumors (ClinicalTrials.gov Identifiers: NCT01362107 and NCT00062049), and an ongoing clinical trial is testing CYT107 in combination with atezolizumab (ClinicalTrials.gov Identifier: NCT03513952) for patients with inoperable urothelial cancer.[95] NT-I7 (efineptakin alfa) is a long-acting Fc-fused human IL-7 developed by NeoImmuneTech, Inc. currently being tested in phase 2 trials in combination with CPIs (ClinicalTrials.gov Identifiers: NCT04332653, NCT04594811, and NCT04984811). Although data on the effect of NT-I7 in TLS development and formation are not available yet, NT-I7 favors peripheral expansion of T memory stem cells (T_{SCM}), increases plasma levels of chemokines such as CCL19, and enhances T-cell infiltration into the tumors, even in immunologically cold tumors like GI cancers.[30–32,96] NT-I7 expands naïve T cells and increases T_{SCM} more than 25-fold,[96] both of which are important mediators of TLS maturation. These studies demonstrate the potential of IL-7–modulating therapies to expand key lymphoid progenitors, promote lymphoid organization, and fuel TLS-associated behavior within tissues. Whether the TLS-inducing capabilities will manifest in clinical trials will soon be evident.

Cellular Therapy Delivers TLS-Inducing Signals into the Tumor Microenvironment

Novel cellular therapies have attempted to leverage cytokine and chemokine signaling to stimulate immunologic reactions against cancer. Genetically modified DCs, mesenchymal stem cells (MSCs), and chimeric antigen receptor T (CAR-T) cells designed to secrete soluble factors such as IL-7, CCL19, or CCL21 have demonstrated the ability to induce T-cell infiltration and even TLS formation within solid tumors in both preclinical and clinical settings.[97–102] CCL19 plays an important role in mediating migration and infiltration of CCR7-expressing naïve and stem-like T and B cells into TLS. DCs, MSCs, and CAR-T cells genetically modified to express CCL19 or CCL21 have demonstrated therapeutic benefit against solid tumors in preclinical and clinical settings.[100,101]

DCs expressing high levels of T-box expressed in T cells (Tbet) have been shown to induce TLS formation in murine models of colorectal cancer.[97–99] These engineered DCs (DC.Tbet) secrete high levels of IFNγ, TNF, and IL-36γ, the latter of which correlates with spontaneous TLS formation in colorectal cancer.[97–99,103] Relative to control DC, DC.Tbet expressed higher levels of CCL19, CCL21, and Ltα1β2 both in vivo and in vitro.[97–99] Vessel formation, B cell infiltration, and enrichment of Tbet-expressing $CD4^+$ T cells were observed following DC.Tbet injection.[97–99] The presence of lymphoid aggregates resembling TLSs were detected by H&E in DC.Tbet-injected tumors, but GC-like organization was not achieved within this model.[97–99]

CAR-T cells expressing CCL19 and IL-7 have been developed to mimic the effects of FRCs on T cell zone generation in lymphoid structures such as TLSs.[100] These CAR-T cells demonstrated efficacy against pancreatic cancer and lung cancer in an IL-7–dependent manner and induced the infiltration and colocalization of DCs and endogenous T cells within tumors.[100] Further, these CAR-T cells induced differentiation of donor and host T cells into central memory cells, characteristic of T-cell differentiation associated with TLSs.[100] A clinical trial is now underway testing CAR-T cells secreting IL-7 and CCL19, targeting glypican-3 or mesothelin, with encouraging reports (ClinicalTrials.gov Identifier: NCT03198546).[101]

Additional strategies, including the concurrent administration of cellular therapies and cytokine-based therapies such as NT-I7, are also being explored. A clinical trial testing the combination of systemic NT-I7 administration after CD19-directed CAR-T cell administration in diffuse-large B cell lymphoma (ClinicalTrials.gov Identifier: NCT05075603) is currently underway. However, the relationship between cellular therapies and TLSs is still poorly understood. As these therapies progress in clinical settings, every effort should be made to understand how infused cellular products stimulate TLS formation or interact with preexisting TLSs.

SUMMARY

Owing to the poor immunogenicity and broad immunosuppressive nature of GI cancers, a better understanding of resistance to immunotherapy and the development of novel therapeutic strategies capable of promoting immune responses is needed. The infiltration and expansion of tumor-specific cytotoxic T cells is the aim of immune-based therapies such as CPIs; however, most patients with GI cancer have resistance to single-agent CPIs. TLSs within solid tumors are a source of cytotoxic T cells, priming naïve T cells to expand and differentiate into tumor-specific effector T cells in situ, and predict response to CPIs in several indications. Increased intratumoral TLSs predict survival in gastric, colon, pancreatic, and hepatic cancer; however, limited studies have explored associations between response to CPIs and TLSs, highlighting a lack of knowledge of adaptive immune responses to GI cancers. A few reports have found that TLSs correlate with response to CPIs in gastric and hepatic cancer, meanwhile TLSs arise in pancreatic and colorectal tumors after treatment with immune therapies in clinical and preclinical models and

these results are summarized here. We review the role of several cytokines (IL-7, Ltα1β2) and chemokines (CCL13, CCL19), which are key for TLS development and represent therapeutic leverage points in GI cancers. IL-7 is of particular interest for therapeutic modulation in patients with cancer, as it plays a role in the initiation, maturation, and sustainment of mature TLSs within solid tumors. Several novel cytokine-focused therapies, like long-acting IL-7, are now in preclinical development or in clinical trials and have demonstrated TLS-inducing capabilities and show potential synergism with CPIs. However, demonstrating the ability of these therapies to enhance TLS formation or maintenance and its relationship with clinical efficacy will be challenging. Determining whether TLSs preexisted or are induced will require the collection of strategic pre-treatment and post-treatment biopsy specimens for longitudinal analysis of TLS formation. Further, establishing standard guidelines to guarantee biopsy quality and standardizing assays for the detection and measurement of TLSs in patient tissues will be crucial for these efforts. As combination therapies continue to be developed in preclinical models of GI cancer, special attention should be given to those combinations that elicit TLS formation.

References

1. Carstens JL, Correa de Sampaio P, Yang D, et al. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. *Nat Commun*. 2017;8:15095.
2. Nywening TM, Wang-Gillam A, Sanford DE, et al. Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: a single-centre, open-label, dose-finding, non-randomised, phase 1b trial. *Lancet Oncol*. 2016;17:651–662.
3. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*. 2017;168:707–723.
4. Jansen CS, Prokhnevska N, Master VA, et al. An intratumoral niche maintains and differentiates stem-like CD8 T cells. *Nature*. 2019;576:465–470.
5. Trub M, Zippelius A. Tertiary Lymphoid Structures as a predictive biomarker of response to cancer immunotherapies. *Front Immunol*. 2021;12:674565.
6. Silina K, Soltermann A, Attar FM, 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;78:1308–1320.
7. Nayar S, Campos J, Chung MM, et al. Bimodal expansion of the lymphatic vessels is regulated by the sequential expression of IL-7 and lymphotoxin alpha1beta2 in newly formed tertiary lymphoid structures. *J Immunol*. 2016;197:1957–1967.
8. Martinet L, Filleron T, Le Guellec S, et al. High endothelial venule blood vessels for tumor-infiltrating lymphocytes are associated with lymphotoxin beta-producing dendritic cells in human breast cancer. *J Immunol*. 2013;191:2001–2008.
9. Muniz LR, Pacer ME, Lira SA, Furtado GC. A critical role for dendritic cells in the formation of lymphatic vessels within tertiary lymphoid structures. *J Immunol*. 2011;187:828–834.
10. Schlosser HA, Thelen M, Lechner A, et al. B cells in esophago-gastric adenocarcinoma are highly differentiated, organize in tertiary lymphoid structures and produce tumor-specific antibodies. *Oncoimmunology*. 2019;8:e1512458.
11. Aoyama S, Nakagawa R, Mule JJ, Mailloux AW. Inducible tertiary lymphoid structures: promise and challenges for translating a new class of immunotherapy. *Front Immunol*. 2021;12:675538.
12. Pipi E, Nayar S, Gardner DH, et al. Tertiary lymphoid structures: autoimmunity goes local. *Front Immunol*. 2018;9:1952.
13. Neyt K, Perros F, GeurtsvanKessel CH, et al. Tertiary lymphoid organs in infection and autoimmunity. *Trends Immunol*. 2012;33:297–305.
14. Vanhersecke L, Brunet M, Guegan JP, 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.
15. Mori T, Tanaka H, Deguchi S, et al. Clinical efficacy of nivolumab is associated with tertiary lymphoid structures in surgically resected primary tumors of recurrent gastric cancer. *PLoS One*. 2022;17:e0262455.
16. Blando J, Sharma A, Higa MG, et al. Comparison of immune infiltrates in melanoma and pancreatic cancer highlights VISTA as a potential target in pancreatic cancer. *Proc Natl Acad Sci U S A*. 2019;116:1692–1697.
17. Kamath SD, Kalyan A, Kircher S, et al. Ipilimumab and gemcitabine for advanced pancreatic cancer: a phase Ib study. *Oncologist*. 2020;25:e808–e815.
18. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509–2520.
19. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 2010;33:828–833.
20. Ueno M, Ikeda M, Morizane C, et al. Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: a non-randomised, multicentre, open-label, phase 1 study. *Lancet Gastroenterol Hepatol*. 2019;4:611–621.
21. Ware MB, El-Rayes BF, Lesinski GB. Mirage or long-awaited oasis: reinvigorating T-cell responses in pancreatic cancer. *J Immunother Cancer*. 2020;8:e001100.
22. Bang Y-J, Ueno M, Malka D, et al. Pembrolizumab (pembro) for advanced biliary adenocarcinoma: results from the KEYNOTE-028 (KN028) and KEYNOTE-158 (KN158) basket studies. *J Clin Oncol*. 2019;37(15 suppl): 4079.
23. Kim RD, Kim DW, Alese OB, et al. A phase II study of nivolumab in patients with advanced refractory biliary tract cancers (BTC). *J Clin Oncol*. 2019;37(15 suppl):4097.
24. Hiraoka N, Ino Y, Yamazaki-Itoh R, et al. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. *Br J Cancer*. 2015;112:1782–1790.
25. Gunderson AJ, Rajamanickam V, Bui C, et al. Germinal center reactions in tertiary lymphoid structures associate with neoantigen burden, humoral immunity and long-

term survivorship in pancreatic cancer. *Oncoimmunology*. 2021;10:1900635.
26. He W, Zhang D, Liu H, et al. The high level of tertiary lymphoid structure is correlated with superior survival in patients with advanced gastric cancer. *Front Oncol*. 2020;10:980.
27. Yamakoshi Y, Tanaka H, Sakimura C, et al. Immunological potential of tertiary lymphoid structures surrounding the primary tumor in gastric cancer. *Int J Oncol*. 2020;57:171–182.
28. Sakimura C, Tanaka H, Okuno T, et al. B cells in tertiary lymphoid structures are associated with favorable prognosis in gastric cancer. *J Surg Res*. 2017;215:74–82.
29. Ding GY, Ma JQ, Yun JP, et al. Distribution and density of tertiary lymphoid structures predict clinical outcome in intrahepatic cholangiocarcinoma. *J Hepatol*. 2022;76:608–618.
30. Lee SW, Choi D, Heo M, et al. hIL-7-hyFc, a long-acting IL-7, increased absolute lymphocyte count in healthy subjects. *Clin Transl Sci*. 2020;13:1161–1169.
31. Kim JH, Kim YM, Choi D, et al. Hybrid Fc-fused interleukin-7 induces an inflamed tumor microenvironment and improves the efficacy of cancer immunotherapy. *Clin Transl Immunol*. 2020;9:e1168.
32. Campian JL, Ghosh S, Kapoor V, et al. Long-acting recombinant human interleukin-7, NT-I7, increases cytotoxic CD8 T cells and enhances survival in mouse glioma models. *Clin Cancer Res*. 2022;28:1229–1239.
33. Derks S, de Klerk LK, Xu X, et al. Characterizing diversity in the tumor-immune microenvironment of distinct subclasses of gastroesophageal adenocarcinomas. *Ann Oncol*. 2020;31:1011–1020.
34. Engelhard VH, Rodriguez AB, Mauldin IS, et al. Immune cell infiltration and tertiary lymphoid structures as determinants of antitumor immunity. *J Immunol*. 2018;200:432–442.
35. Li H, Liu H, Fu H, et al. Peritumoral tertiary lymphoid structures correlate with protective immunity and improved prognosis in patients with hepatocellular carcinoma. *Front Immunol*. 2021;12:648812.
36. Ruffin AT, Cillo AR, Tabib T, et al. B cell signatures and tertiary lymphoid structures contribute to outcome in head and neck squamous cell carcinoma. *Nat Commun*. 2021;12:3349.
37. Calderaro J, Petitprez F, Becht E, et al. Intra-tumoral tertiary lymphoid structures are associated with a low risk of early recurrence of hepatocellular carcinoma. *J Hepatol*. 2019;70:58–65.
38. Ghidini M, Lampis A, Mirchev MB, et al. Immune-based therapies and the role of microsatellite instability in pancreatic cancer. *Genes (Basel)*. 2020;12:33.
39. Maby P, Bindea G, Mlecnik B, Galon J. License to kill: microsatellite instability and immune contexture. *Oncoimmunology*. 2021;10:1905935.
40. Puliga E, Corso S, Pietrantonio F, Giordano S. Microsatellite instability in gastric cancer: between lights and shadows. *Cancer Treat Rev*. 2021;95:102175.
41. Layke JC, Lopez PP. Gastric cancer: diagnosis and treatment options. *Am Fam Physician*. 2004;69:1133–1140.
42. Liu D, Lu M, Li J, et al. The patterns and timing of recurrence after curative resection for gastric cancer in China. *World J Surg Oncol*. 2016;14:305.
43. Rodriquenz MG, Roviello G, D'Angelo A, et al. MSI and EBV positive gastric cancer's subgroups and their link with novel immunotherapy. *J Clin Med*. 2020;9:1427.
44. Fuchs CS, Doi T, Jang RW, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. *JAMA Oncol*. 2018;4:e180013.
45. Mori T, Tanaka H, Suzuki S, et al. Tertiary lymphoid structures show infiltration of effective tumor-resident T cells in gastric cancer. *Cancer Sci*. 2021;112:1746–1757.
46. Du F, Liu Y. Predictive molecular markers for the treatment with immune checkpoint inhibitors in colorectal cancer. *J Clin Lab Anal*. 2022;36:e24141.
47. Ding Y, Weng S, Li X, et al. General treatment for metastatic colorectal cancer: from KEYNOTE 177 study. *Transl Oncol*. 2021;14:101122.
48. Sun BL. Current microsatellite instability testing in management of colorectal cancer. *Clin Colorectal Cancer*. 2021;20:e12–e20.
49. Kim JH, Seo MK, Lee JA, et al. Genomic and transcriptomic characterization of heterogeneous immune subgroups of microsatellite instability-high colorectal cancers. *J Immunother Cancer*. 2021;9:e003414.
50. Le DT, Kim TW, Van Cutsem E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol*. 2020;38:11–19.
51. Maoz A, Dennis M, Greenson JK. The crohn's-like lymphoid reaction to colorectal cancer-tertiary lymphoid structures with immunologic and potentially therapeutic relevance in colorectal cancer. *Front Immunol*. 2019;10:1884.
52. Rozek LS, Schmit SL, Greenson JK, et al. Tumor-infiltrating lymphocytes, Crohn's-like lymphoid reaction, and survival from colorectal cancer. *J Natl Cancer Inst*. 2016;108:djw027.
53. Zhao Z, Ding H, Lin ZB, et al. Relationship between tertiary lymphoid structure and the prognosis and clinicopathologic characteristics in solid tumors. *Int J Med Sci*. 2021;18:2327–2338.
54. Sautes-Fridman C, Lawand M, Giraldo NA, et al. Tertiary lymphoid structures in cancers: prognostic value, regulation, and manipulation for therapeutic intervention. *Front Immunol*. 2016;7:407.
55. Chalabi M, Fanchi LF, Dijkstra KK, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med*. 2020;26:566–576.
56. Yamaguchi K, Ito M, Ohmura H, et al. Helper T cell-dominant tertiary lymphoid structures are associated with disease relapse of advanced colorectal cancer. *Oncoimmunology*. 2020;9:1724763.
57. Luchini C, Brosens LAA, Wood LD, et al. Comprehensive characterisation of pancreatic ductal adenocarcinoma with microsatellite instability: histology, molecular pathology and clinical implications. *Gut*. 2021;70:148–156.
58. Lupinacci RM, Goloudina A, Buhard O, et al. prevalence of microsatellite instability in intraductal papillary mucinous neoplasms of the pancreas. *Gastroenterology*. 2018;154:1061–1065.
59. Kuwabara S, Tsuchikawa T, Nakamura T, et al. Prognostic relevance of tertiary lymphoid organs following neoadjuvant chemoradiotherapy in pancreatic ductal adenocarcinoma. *Cancer Sci*. 2019;110:1853–1862.
60. Lee YT, Wang JJ, Luu M, et al. Comparison of clinical features and outcomes between intrahepatic cholangio-

carcinoma and hepatocellular carcinoma in the United States. *Hepatology*. 2021;74:2622–2632.
61. Ware MB, Zaidi MY, Yang J, et al. Suppressive myeloid cells are expanded by biliary tract cancer-derived cytokines in vitro and associate with aggressive disease. *Br J Cancer*. 2020;123:1377–1386.
62. Refolo MG, Messa C, Guerra V, et al. Inflammatory mechanisms of HCC development. *Cancers (Basel)*. 2020;12:641.
63. Wen S, Chen Y, Hu C, et al. Combination of tertiary lymphoid structure and neutrophil-to-lymphocyte ratio predicts survival in patients with hepatocellular carcinoma. *Front Immunol*. 2021;12:788640.
64. Garnelo M, Tan A, Her Z, et al. Interaction between tumour-infiltrating B cells and T cells controls the progression of hepatocellular carcinoma. *Gut*. 2017;66:342–351.
65. Ho WJ, Zhu Q, Durham J, et al. Neoadjuvant cabozantinib and nivolumab converts locally advanced HCC into resectable disease with enhanced antitumor immunity. *Nat Cancer*. 2021;2:891–903.
66. Guyot C, Lepreux S, Combe C, et al. Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved. *Int J Biochem Cell Biol*. 2006;38:135–151.
67. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer*. 2019;19:307–325.
68. Gago da Graca C, van Baarsen LGM, Mebius RE. Tertiary lymphoid structures: diversity in their development, composition, and role. *J Immunol*. 2021;206:273–281.
69. Cupedo T, Crellin NK, Papazian N, et al. Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells. *Nat Immunol*. 2009;10:66–74.
70. van de Pavert SA, Olivier BJ, Goverse G, et al. Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. *Nat Immunol*. 2009;10:1193–1199.
71. Yoshida H, Honda K, Shinkura R, et al. IL-7 receptor alpha+ CD3(-) cells in the embryonic intestine induces the organizing center of Peyer's patches. *Int Immunol*. 1999;11:643–655.
72. Yoshida H, Naito A, Inoue J, et al. Different cytokines induce surface lymphotoxin-alphabeta on IL-7 receptor-alpha cells that differentially engender lymph nodes and Peyer's patches. *Immunity*. 2002;17:823–833.
73. Ohl L, Henning G, Krautwald S, et al. Cooperating mechanisms of CXCR5 and CCR7 in development and organization of secondary lymphoid organs. *J Exp Med*. 2003;197:1199–1204.
74. Sudhamsu J, Yin J, Chiang EY, et al. Dimerization of LTbetaR by LTalpha1beta2 is necessary and sufficient for signal transduction. *Proc Natl Acad Sci U S A*. 2013;110:19896–19901.
75. Tang H, Zhu M, Qiao J, Fu YX. Lymphotoxin signalling in tertiary lymphoid structures and immunotherapy. *Cell Mol Immunol*. 2017;14:809–818.
76. Iolyeva M, Aebischer D, Proulx ST, et al. Interleukin-7 is produced by afferent lymphatic vessels and supports lymphatic drainage. *Blood*. 2013;122:2271–2281.
77. Ansel KM, Ngo VN, Hyman PL, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature*. 2000;406:309–314.
78. Luther SA, Ansel KM, Cyster JG. Overlapping roles of CXCL13, interleukin 7 receptor alpha, and CCR7 ligands in lymph node development. *J Exp Med*. 2003;197:1191–1198.
79. Li X, Wan Z, Liu X, et al. A 12-chemokine gene signature is associated with the enhanced immunogram scores and is relevant for precision immunotherapy. *Med Oncol*. 2022;39:43.
80. Mackley EC, Houston S, Marriott CL, et al. CCR7-dependent trafficking of RORgamma(+) ILCs creates a unique microenvironment within mucosal draining lymph nodes. *Nat Commun*. 2015;6:5862.
81. Wu BX, Zhao LD, Zhang X. CXCR4 and CXCR5 orchestrate dynamic germinal center reactions and may contribute to the pathogenesis of systemic lupus erythematosus. *Cell Mol Immunol*. 2019;16:724–726.
82. Peske JD, Thompson ED, Gemta L, et al. Effector lymphocyte-induced lymph node-like vasculature enables naive T-cell entry into tumours and enhanced anti-tumour immunity. *Nat Commun*. 2015;6:7114.
83. Thompson ED, Enriquez HL, Fu YX, Engelhard VH. Tumor masses support naive T cell infiltration, activation, and differentiation into effectors. *J Exp Med*. 2010;207:1791–1804.
84. Schrama D, Voigt H, Eggert AO, et al. Immunological tumor destruction in a murine melanoma model by targeted LTalpha independent of secondary lymphoid tissue. *Cancer Immunol Immunother*. 2008;57:85–95.
85. Jiao S, Xiong Q, Yan M, et al. Intratumor expanded T cell clones can be non-sentinel lymph node derived in breast cancer revealed by single-cell immune profiling. *J Immunother Cancer*. 2022;10:e003325.
86. Kroncke R, Loppnow H, Flad HD, Gerdes J. Human follicular dendritic cells and vascular cells produce interleukin-7: a potential role for interleukin-7 in the germinal center reaction. *Eur J Immunol*. 1996;26:2541–2544.
87. Koning JJ, Rajaraman A, Reijmers RM, et al. Development of follicular dendritic cells in lymph nodes depends on retinoic acid-mediated signaling. *Development*. 2021;148:dev199713.
88. Knop L, Deiser K, Bank U, et al. IL-7 derived from lymph node fibroblastic reticular cells is dispensable for naive T cell homeostasis but crucial for central memory T cell survival. *Eur J Immunol*. 2020;50:846–857.
89. Helmink BA, Reddy SM, Gao J, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature*. 2020;577:549–555.
90. Cabrita R, Lauss M, Sanna A, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature*. 2020;577:561–565.
91. Barone F, Bombardieri M, Manzo A, et al. Association of CXCL13 and CCL21 expression with the progressive organization of lymphoid-like structures in Sjogren's syndrome. *Arthritis Rheum*. 2005;52:1773–1784.
92. Astorri E, Bombardieri M, Gabba S, et al. Evolution of ectopic lymphoid neogenesis and in situ autoantibody production in autoimmune nonobese diabetic mice: cellular and molecular characterization of tertiary lymphoid structures in pancreatic islets. *J Immunol*. 2010;185:3359–3368.
93. Seo YB, Im SJ, Namkoong H, et al. Crucial roles of interleukin-7 in the development of T follicular helper cells and in the induction of humoral immunity. *J Virol*. 2014;88:8998–9009.
94. Beq S, Rozlan S, Gautier D, et al. Injection of glycosylated recombinant simian IL-7 provokes rapid and massive T-

cell homing in rhesus macaques. *Blood*. 2009;114:816–825.
95. Pachynski RK, Morishima C, Szmulewitz R, et al. IL-7 expands lymphocyte populations and enhances immune responses to sipuleucel-T in patients with metastatic castration-resistant prostate cancer (mCRPC). *J Immunother Cancer*. 2021;9:e002903.
96. Kim R, Barve M, Mamdani M, et al. 404 Initial biomarker and clinical data of a phase 2a study of NT-I7, a long-acting interleukin-7, plus pembrolizumab: cohort of subjects with checkpoint inhibitor-naive advanced MSS-colorectal cancer. *J Immunother Cancer*. 2021;9: DOI: 10.1136/jitc-2021-SITC2021.404
97. Lipscomb MW, Chen L, Taylor JL, et al. Ectopic T-bet expression licenses dendritic cells for IL-12-independent priming of type 1 T cells in vitro. *J Immunol*. 2009;183:7250–7258.
98. Weinstein AM, Chen L, Brzana EA, et al. Tbet and IL-36gamma cooperate in therapeutic DC-mediated promotion of ectopic lymphoid organogenesis in the tumor microenvironment. *Oncoimmunology*. 2017;6:e1322238.
99. Chen L, Fabian KL, Taylor JL, Storkus WJ. Therapeutic use of dendritic cells to promote the extranodal priming of anti-tumor immunity. *Front Immunol*. 2013;4:388.
100. Adachi K, Kano Y, Nagai T, et al. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol*. 2018;36:346–351.
101. Pang N, Shi J, Qin L, et al. IL-7 and CCL19-secreting CAR-T cell therapy for tumors with positive glypican-3 or mesothelin. *J Hematol Oncol*. 2021;14:118.
102. Iida Y, Yoshikawa R, Murata A, et al. Local injection of CCL19-expressing mesenchymal stem cells augments the therapeutic efficacy of anti-PD-L1 antibody by promoting infiltration of immune cells. *J Immunother Cancer*. 2020;8e000582.
103. Weinstein AM, Giraldo NA, Petitprez F, et al. Association of IL-36gamma with tertiary lymphoid structures and inflammatory immune infiltrates in human colorectal cancer. *Cancer Immunol Immunother*. 2019;68:109–120.