T cell exhaustion initiates tertiary lymphoid structures and turbocharges cancer-immunity cycle

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Summary

Immune therapies represented by immune checkpoint blockade (ICB) have significantly transformed cancer treatment. However, the effectiveness of these treatments depends on the status of T cells. T cell exhaustion, characterized by diminished effector function, increased expression of co-inhibitory receptors, and clonal deletion, emerges as a hypofunctional state resulting from chronic exposure to antigens, posing an obstacle to ICB therapy. Several studies have deeply explored T cell exhaustion, providing innovative insights and correlating T cell exhaustion with tertiary lymphoid structures (TLS) formation. TLS, lymphocyte aggregates formed in non-lymphoid tissues amid chronic inflammation, serve as pivotal reservoirs for anti-tumour immunity. Here, we underscore the pivotal role of T cell exhaustion as a signalling mechanism in reinvigorating anti-tumour immunity by turbocharging cancer-immunity (CI) cycle, particularly when tumour becomes unmanageable. Building upon this concept, we summarize emerging immunotherapeutic strategies aimed at enhancing the response rate to ICB therapy and improving patient prognosis.

Keywords: T cell exhaustion; Tertiary lymphoid structure; Immunotherapy; Cancer

Introduction

Recent research has indicated that the infiltration of immune cells within the tumour immune microenvironment (TIME) is linked to a favourable prognosis.[1,2] The advent of immune checkpoint blockade (ICB) therapies[3–6] and adoptive cell therapy (ACT)[7,8] have garnered significant interest and have shown promising clinical outcomes. These advancements have sparked interest in the study of T cell exhaustion, which is a kind of dysfunction primarily induced by persistent antigen exposure.[8–10] Exhausted T cells (Tex) exhibit diminished effector functions, increased expression of co-inhibitory receptors, and clonal deletion.[9–12] Such impairments are believed to impede the efficacy of immunotherapies. Recently, several studies have discovered that T cells with different functional impairments show variations in effector capacity. The progression of T cell exhaustion encompasses progenitor exhausted T cells (Tex^{prog}) with stem-like characteristics and terminally exhausted T cells (Tex^{term}) that exhibit loss of function and proliferative capacity.[13,14] Notably, upon transition into an exhausted state, T cells express an increasing amount of chemokine C-X-C motif ligand 13 (CXCL13).[15–17] CXCL13 plays a significant role in the organization and formation of tertiary lymphoid structures (TLS),[17–19] leading to the question of whether T cell exhaustion is in some way linked with the formation of TLS.

TLS serve as pivotal immune centres within tumours, orchestrating antigen presentation and fostering lymphocyte activation, which is essential for anti-tumour responses. TLS are organized lymphocytic aggregates, comprising B cells, T cells, dendritic cells (DCs), fibroblastic reticular cells (FRCs) and high endothelial venules (HEVs), which arise in non-lymphoid tissues under chronic inflammatory conditions.[19–23] TLS differ from secondary lymphoid organs (SLOs), such as lymph nodes (LNs), in lacking a capsule and possessing unique functions and characteristics.[24] Within tumours, TLS function as the front line of anti-tumour immune response. They facilitate

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the rapid presentation of antigens to T cells by DCs and provide a niche conducive to the activation, proliferation, and differentiation of T and B cells.[22] A growing body of evidence suggests that the presence of TLS within tumours predicts a favourable prognosis for cancer patients,[22] and is strongly associated with the response to immunotherapy.[25–29]

The concept of the cancer-immunity (CI) cycle provided a framework for understanding anti-tumour immunity.[30] This theory elucidates that T cells within tumours do not operate in isolation but are involved in a series of iterative events.[30] The CI cycle offers a crucial perspective, suggesting that successful anti-tumour immunity requires the ability to self-amplify during the response process. Recent viewpoints have incorporated TLS into the CI cycle as a "subcycle". Mellman et al. propose that DCs within tumours can be localized within TLS, thereby initiating T cell proliferation and differentiation, and forming localized TIME eddy within the CI cycle.[31] With advancing research on T cell heterogeneity, increasing evidence indicates that T cells can act as catalysts for these eddies. T cells, especially Tex^{term}, may initiate more potent anti-tumour immunity in situ by promoting the formation of TLS.

In this review, we discuss the pivotal alterations in the TIME during the process of T cell exhaustion, along with its potential association with the development of TLS (Fig. 1). We emphasize the possibility of Tex^{term} serving as a "call-for-help" to rebuild the anti-tumour immune defence line. We focused particularly on $CD8^+$ Tex^{term}, owing to their significant role during ICB therapy. This will prompt a reassessment of the roles of T cell exhaustion and TLS in anti-tumour immunity and lay a groundwork for the advancement of novel methods based on reversing T cell exhaustion and inducing TLS formation. This could enhance the response of cancer patients to ICB therapy and improve prognosis.

The role of T cell exhaustion in anti-tumour immunity

T cell exhaustion delineates the diminished response of T cells to persistent antigenic stimulation.[9,13,32–36] The characteristics of Tex include a reduction in cytokine production, inhibitory receptors (PD-1, CTLA4, and LAG3), the immunosuppressive enzyme CD39 and CXCL13.[13,15] In the late 20th century, researchers had already described the hyporesponsive $CD8^+$ T cell state observed in lymphocytic choriomeningitis viral (LCMV) infections as "exhaustion".[37–39] The differentiation process of T cell exhaustion is accompanied by increased expression of inhibitory receptors and CXCL13, alongside decreased effector functionality.[15,40] CXCL13 was initially identified as a molecular marker closely associated with the exhaustion of $CD8^+$ T cells.[15] However, subsequent studies have demonstrated that CXCL13 is a molecular marker of tumour-specific T cells.[41–43] Furthermore, recent research has shown that $CXCL13^+CD8^+$ Tex can effectively predict the efficacy of immunotherapy.[44] These findings indicate the potent immunosuppressive mechanisms within the TIME that rapidly drive the exhaustion of tumour-specific $CD8^+$ T cells. While Tex^{term} are often seen as nonfunctional, recent studies have shown that even Tex^{term} can produce effector molecules at the transcriptional level and proliferate in an antigen-dependent manner, potentially contributing to their numerical dominance in the TIME.[40] Historically, the presence of Tex has been considered a major obstacle to successful immunotherapy. Research has concentrated on rejuvenating Tex function through ICB and other immunotherapeutic strategies. However, Tex^{term} were resistant to such interventions due to epigenetic modifications that solidify their state.[45] Recently, Chow et al. proposed a balanced perspective on T cell exhaustion.[13] They propose that T cell exhaustion is inevitable in the case of persistent tumour antigens.[13] Consequently, it is imperative to improve the efficacy of tumour immunotherapy strategies through an in-depth investigation into the mechanisms underlying T cell exhaustion.

During the exhaustion process, the cytotoxic functionality and clonal proliferation ability of T cells gradually diminish, ultimately leading to a loss of effector capability. A subset of Tex^{prog}, expressing the T cell factor 1 (TCF1), exhibits self-renewal and differentiation capabilities but has weaker cytotoxicity.[46] These cells are considered to be reservoirs for Tex^{term}, capable of replenishing tumour-specific T cells, thereby maintaining an immune defence composed of tumour-specific T cells.[47] According to recent views, the effectiveness of ICB treatment relies on the peripheral expansion and tumour infiltration of Tex^{prog}.[13] Interestingly, Tex^{prog} also express C-X-C motif chemokine receptor 5 (CXCR5) and are recruited and stimulated by CXCL13,[48] explaining the abundance of Tex^{prog} in the Tex^{term} compartment.[40] In both mouse models and humans, enrichment of Tex^{prog} in LNs and TLS has been observed, indicating that sustaining Tex^{prog} requires supportive interactions with other immune cells found in these locations.[40,49]

Tex^{term} may be an inducer of intratumoral TLS. Traditionally, the formation of SLOs is believed to depend on lymphoid tissue organizer (LTo) and lymphoid tissue inducer (LTi) cells.[40] The formation of TLS is similarly believed to necessitate these or analogous cells with comparable functions.[20] We propose that Tex^{term} potentially drive the activation of LTo cells, thus instigating the formation of TLS. However, this assumption cannot be established based solely on the expression of CXCL13, as tissue-specific induction of CXCL13 only leads to B cell aggregates devoid of the

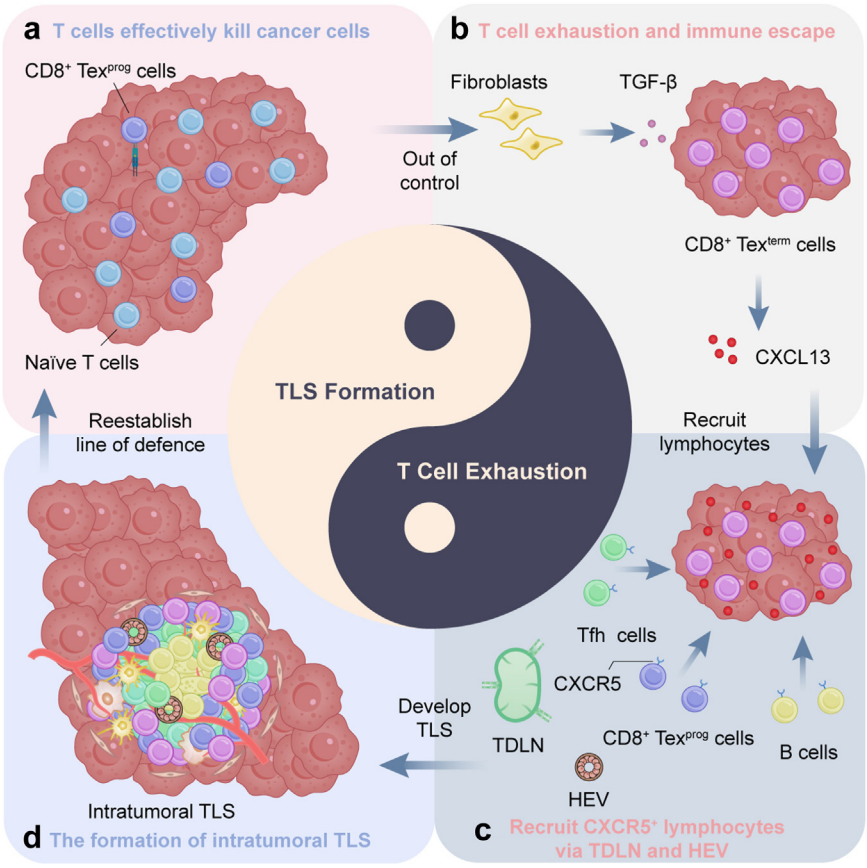

***Fig. 1:* Schematic illustration of the immune cycle between T cell exhaustion and TLS formation**. a. In TIME, naïve T cells are stimulated and begin to undergo exhaustion. b. As T cells progress from Tex^{prog} to Tex^{term}, their effector functions are severely impaired, losing the ability to suppress tumour growth. Under the action of TGF-β secreted by CAFs, Tex^{term} begins to highly express CXCL13. c. CXCL13 recruits $CXCR5^+$ B cells, Tfh cells and Tex^{prog} through tumour-draining lymph nodes (TDLNs) or HEVs. d. $CXCR5^+$ lymphocytes organize TLS in tumours. Mature TLS reestablish the anti-tumour immune defence line and achieve the control over tumour growth.

follicular dendritic cell (FDC) network.[50] Consequently, discussions on TLS formation should be considered within the wider context of T cell exhaustion.

Exhausted T cells at the crossroads of effector responses and tolerance

Tex may serve as a trigger to reactivate immune defence. The notion of cancer immunoediting suggests that tumours progressively construct an immunosuppressive TIME to ensure their survival, leading to the gradual diminishment of T cell effectiveness.[51] Although the emergence of Tex^{term} indicates a failure to control the tumour, it may represent more than a mere phenomenon. Instead, Tex^{term} could serve as a "trigger" to reconstruct immune defence through alternative methods, with the formation of TLS potentially being one such approach. Herein, we have summarized the various states of T cells associated with TLS formation (Table 1). Building on this, we organized evidence supporting the involvement of T cell exhaustion in the initiation of TLS formation.

Exhausted T cells initiate and amplify the CXCL13-CXCR5 signalling axis

As a critical molecular factor in the formation of TLS, CXCL13 recruits $CXCR5^+$ immune cells within the TIME.[62] In LNs, the CXCL13-CXCR5 signalling axis is crucial for B cell homing.[63] Similarly, within TLS, CXCL13 mediates the recruitment and positioning of B cells. A recent study identified a subset of $LAYN^+CD8^+$ T cells producing CXCL13 that drive intratumoral TLS formation in colorectal cancer.[52] This subset was previously categorized within Tex.[56] However, Tex are not the sole source of CXCL13. Additionally, CXCL13 within TLS emanates from cancer-associated fibroblasts (CAFs) and follicular helper T (Tfh) cells.[16,64] Tfh cells aid B cells in iteratively mutating their B cell receptors (BCRs), a mechanism that enhances the affinity of antibody responses and promotes the development of germinal

Tumour type	Annotation	Gene signature	Expression of inhibitory receptor	Differentiation trajectory	Functional characteristics	Ref
CD8$^+$ T cells						
HNSCC	Exhausted T cells	*CCL4L2, GZMB, CXCL13, CCL4, IFNG*	NA	CD8$^+$ effector T cells	Cytotoxicity	16
CRC	CD8-LAYN (Exhausted T cells)	*CXCL13*	NA	NA	Chemotactic effects on CD20$^+$CXCR5$^+$ B cells	52
Breast cancer	Exhausted T cells	*CXCL13, GZMB, TNFRSF9, CTLA4, LAG3, PDCD1, HAVCR2, VCAM1*	High	Cytotoxic T cells	Systematic enrichment of immunomodulatory, chemoattractive, and cytokine signalling	53
	Progenitor T cells	*PDCD1, TCF7, CD44*	High	Naïve/early activated T cells and terminal exhausted T cells	Enrichment in the HEVs	54
GC	CD8_Tex	*HAVCR2, GZMA, GZMH, CTLA4, KLRC1, CXCL13*	High	Tissue-resident memory CD8$^+$ T cells	Low cytotoxicity	55
HCC	CD8-LAYN	*CTLA4, PDCD1, HAVCR2, CXCL13, LAYN*	High	CD8-GZMK (represent cells in a transition state from effector to exhausted T cells)	NA	56
	CD8-GZMK	*GZMK, TCF7, GNLY, KLRG1, PDCD1, TIGHT*	Intermediate	Effector T cells and exhausted T cells	NA	
	Exhausted CTLs	*CTLA4, CD27, PDCD1, CXCL13, LTB, GPR183, IL7R, CCR7*	High	Pre-exhausted CTLs	Higher abundances in HBV/HCV-related HCC	57
LUAD	CD103$^+$ T_{RM} cells	*PDCD1, CTLA4, LAG3, HAVCR2, CD96, TNFRSF9, CXCL13*	High	NA	Surrounds the TLS structure and is related to the TLS density	58
	CD8-dysfunctional	*LAG3, TRAC, GZMK, GZMB, CXCL13, CD27*	High	CD8$^+$ T_{RM}	Concentrated in the TLS together with Tfh cells and B cells	59
Melanoma	naïve and/or memory-like T cells	*TCF7, IL7R*	NA	NA	Related to B cell enrichment	25
	CD8$^+$ T cells	*CXCL13, LAT, SKAP1*	NA	CD8$^+$ T cells in TLS with rich B cells	The main source of TLS gene signature (CXCL13)	
CD4$^+$ T cells						
GC	CD4_Tfh-CXCR5	*CXCR5, CXCL13*	Low	Naïve CD4$^+$ T cells	NA	55
	CD4_Tfh-CXCL13	*CXCR5, CCR7, BCL6, IRF4, CXCL13*	High	CXCR5$^+$CD4$^+$ Tfh cells	NA	
HCC	CD4-CXCL13	*CXCL13, PDCD1, CTLA4, TIGIT*	High	T helper cells	Exhausted	56
	Central memory T cells	*CCR7, IL7R, ANXA1, LTB, CXCR4*	Low	Cytotoxic T lymphocytes	Enrichment in the early TLS	57
NPC	PD-1$^+$CXCR5$^-$ Tfh cells	*PDCD1, IFNG, CXCL13*	High	NA	Chemotactic effects on CXCR5$^+$ B cells	60
HNSCC	Th1 cells	*CXCL13, IFNG, BHLHE40*	NA	Effector memory T cells	Chemotactic effects on CXCR5$^+$ cells, associated with favourable prognosis	16
	Tfh cells	*CXCR5, CD40LG, PDCD1, ICA1, ICOS*	NA	Naïve T cells, resident memory T cells, effector memory T cells, and Tregs	Associated with favourable prognosis, activation, and maturation of B cells in TLS	
Breast cancer	Tfh cells	*CXCL13, NR3C1, NMB, PDCD1*	High	NA	Correlated with plasma cell but not B cell	53
	Central memory T cells	*Cd40lg, Il7r, Cd69, Ccr7, Sell*	NA	NA	Interaction with B cells through *Cd40lg* and *Traf3*	61
LUAD	CD4-Tfh like	*CXCL13, RGS1, TRAC, CTLA4, DUSP4, IL6ST, TIGIT*	High	NA	Concentrated in the TLS together with dysfunctional CD8$^+$ T cells and B cells	59

Abbreviations are as follows: Ref, reference; HNSCC, head and neck squamous cell carcinoma; CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; NA, not applicable; LUAD, lung adenocarcinoma; NPC, nasopharyngeal carcinoma; T_{RM}, tissue-resident memory T cell.

Table 1: **T cell states within the TIME associated with TLS formation.**

centres (GCs).[65,66] While acknowledging the contributions of CAFs and Tfh cells to TLS formation, we regard the CXCL13 expressed by these cells dose not initiate, but rather amplifies the CXCL13-CXCR5 signalling axis. Recent research has provided support for this hypothesis. Liu et al. found that during the formation of TLS, CXCL13 primarily originates from CD8$^+$ Tex and Tfh cells.[59] Further in-depth studies have discovered that

$CD8^+$ Tex cells expressing CXCL13, along with conventional $CD4^+$ T cells, are identified as early as in the precursor TLS stage (they define TLS as precursor, early, and mature).[59] However, Tfh cells expressing CXCL13 massively infiltrate during the early TLS stage.[59] Additionally, Tfh cells play a more significant role in promoting the differentiation of B cells.[59] This finding suggests that the role of $CD8^+$ Tex might be inclined toward the early phase in this signalling axis, whereas Tfh cells are more inclined to amplify this signalling axis.

There is a unique crosstalk between $CD8^+$ Tex and Tfh cells. On the one hand, $CD8^+$ Tex can produce CXCL13, which attracts Tfh cells and $CXCR5^+$ B cells to home in the tumour, thus forming TLS. A study has shown that $CXCR5^+$ Tfh cells are considered precursors of $CXCL13^+$ Tfh cells in the differentiation spectrum.[55] On the other hand, Tfh cells within the tumour can prevent Tex^{prog} from developing into Tex^{term} in an interleukin-21 (IL-21) dependent manner,[67] which explains how TLS provides protection for a large number of effector T cells internally. In intratumoral TLS, the greater value of Tfh cells lies in promoting B cell recruitment and differentiation, which will be elaborated further in the subsequent sections. A recent study in pancreatic ductal adenocarcinoma (PDAC) showed that $CD8^+$ and $CD4^+$ T cell expression of CXCL13 depended on two major factors: chronic T cell receptor (TCR) activation and transforming growth factor-β (TGF-β) signaling.[68] In this study, TGF-β mainly came from fibroblasts, which not only played a core role in promoting T cell production of CXCL13, but were also enriched in tumours with TLS.[68] However, TGF-β showed an opposite role in controlling Tfh cells differentiation. TGF-β could limit Tfh cells generation by inhibiting TCR signaling.[69] However, TGF-β promoted Tfh cells differentiation and spontaneous formation of TLS by mediating the silencing of SATB1.[70] In addition, TGF-β can mediate the inhibition of IL-2R expression to block the IL-2 signal transduction of $CD4^+$ T cells.[69] IL-2 mediates multiple pleiotropic effects, including the enhancement of $CD8^+$ T cell proliferation and effector functions, as well as the inhibition of $CD4^+$ T cell differentiation into Tfh cells.[71] A recent study in breast cancer revealed that the deprivation of IL-2 is critical for the production of CXCL13.[64] This further corroborates our view, as IL-2 secretion decreases or is absent when T cell function is exhausted.[72] Moreover, a recent study in colorectal cancer used TGF-β1 and IL-2 to stimulate $CD8^+$ T cells, resulting in a marked increase in the expression of CXCL13.[52] This also further suggests that the CXCL13-CXCR5 signalling axis is the beginning of a new round of immunity in a highly immunosuppressive environment. Here, we summarize the process of T cell exhaustion initiated by the CXCL13-CXCR5 signalling axis to initiate TLS formation (Fig. 2).

However, the CXCL13-CXCR5 axis does not always have the ability to exert anti-tumour effects. In contrast, in most cases, the CXCL13-CXCR5 axis participates in promoting tumorigenesis and progression.[73] In most solid tumours, the CXCL13-CXCR5 axis induces IL-10 to suppress anti-tumour immunity and recruits MDSC and Treg cells to promote tumour invasion.[73] This may be a phenomenon when T cells are still in a progressive exhaustion process. In addition, the regulation of TLS formation by cytokines is very complex, and at least seven major types of cytokines are involved in the TLS formation.[19] Whether Tex are involved in more signal axes is not clear and needs further exploration.

The formation of HEV is associated with the T cell exhaustion

HEV is the primary pathway for peripheral lymphocytes to infiltrate into the tumour. In mouse models, lymphocytes mainly enter tumours through tumour-associated HEV in both baseline and PD-1/CTLA-4 antibody therapy.[74] This kind of HEV is derived from tumour-associated endothelial cells and is the main site for lymphocyte stagnation and extravasation into the tumor.[54,74] A recent study using single-cell transcriptomics verified the specific distribution of $CD8^+$ T cells and found that Tex^{prog} were concentrated near HEV, forming a lymphocyte niche and promoting the proliferation and cytotoxic $CD8^+$ T cells.[54]

There are many differences between the spontaneous formation of tumour-associated HEV and lymph node-associated HEV. Current research suggests that tumour-associated HEV involves multiple developmental steps. First, immature MECA-79^+ HEV-like vessels are produced by tumour necrosis factor receptor (TNFR) signalling, which promotes inflammation, while lymphotoxin β-receptor (LTβR) signalling is more involved in HEV maturation and maintenance.[75] Interestingly, TGF-β is critical for CXCL13 production,[60,76,77] indicating that functional $CD8^+$ T cells are necessary for both formation of HEV and TLS at the initial phases of tumour progression.[78]

Exhausted T cells eventually activate stronger anti-tumour immunity through Tfh and B cells

$CD8^+$ T cells have long been the focal point of anti-tumour immunity. In addition to $CD8^+$ T cells, some $CD4^+$ T cells that have a specific response to tumour antigens also exhibit characteristics of high expression of exhaustion markers such as CXCL13.[41,42,79] However, a growing amount of studies have focused on the roles of $CD4^+$ T cells and B cells in anti-tumour immunity. The discovery and in-depth study of Tfh cells have pushed these two cells to a new height. Notably, the recruitment and differentiation of Tfh and B cells exhibit a strong association with the T cell exhaustion process.

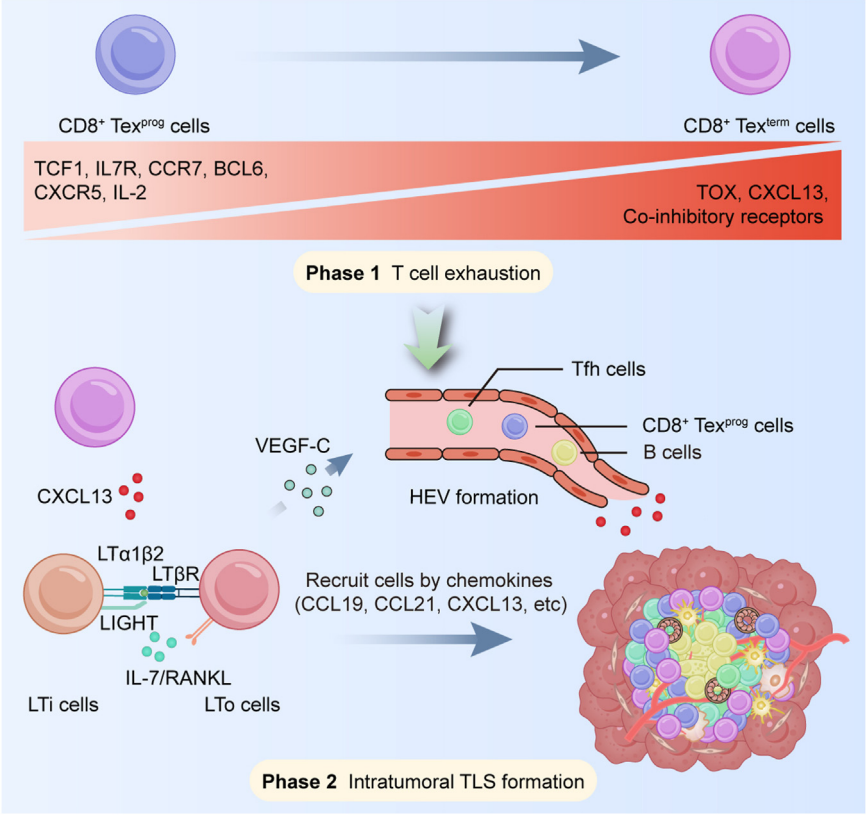

***Fig. 2:* T cell exhaustion drives the formation of intratumoral TLS**. T cells gradually lose the expression of TCF1, IL7R, CCR7, BCL6, CXCR5, and IL-2 during exhaustion, while upregulating the expression of TOX, CXCL13, and co-inhibitory receptors. Tex recruits and activates LTi cells by secreting CXCL13, inducing LTi cells to express LTα1β2, LIGHT, and IL-7, which in turn induces LTo cells to release VEGF-C and chemokines. LTo cells drive the formation of HEV and the recruitment of lymphocytes, thus forming TLS within the tumour.

Tfh cells are a special subset of CD4$^+$ T cells that exhibit elevated levels of CXCR5, inducible co-stimulatory molecule (ICOS), PD-1, IL-21, and B cell lymphoma 6 (BCL-6).[80] Tfh cells support the formation of GC, regulate B cell clonal selection and differentiation, and control antibody affinity maturation and memory.[81] Tfh cells possess the capacity to differentiate into FOXP3$^+$ cells, and are the main source of T follicular regulatory (Tfr) cells in SLO.[82] Notably, Tfr cells in SLO retain the characteristics of Tfh cells secreting IL-21 and GC localization, and their differentiation shows IL-2 dose dependence.[82] Tfh cells can undergo transformation into Tfr cells with lower doses of IL-2, whereas higher doses of IL-2 significantly hinder the development of mouse Tfr cells.[82,83] It is noteworthy that recent studies have observed similar phenomena within TLS. The research conducted by Liu et al. has confirmed that Tfh cells, B cells, and Tex^{term} progressively increase during tumour progression and collectively constitute TLS.[59] In TLS, the abundance of CD8$^+$ Tex^{term} is significantly correlated with Tfh cells. Multiplexed immunohistochemistry also showed that CD8$^+$ Tex^{term} and Tfh cells colocalize within TLS.[59] This study unveils an intriguing perspective that T cell exhaustion might promote the formation of TLS by influencing the recruitment of Tfh and B cells.

The crosstalk between T cell exhaustion and TLS formation guides new immunotherapeutic strategies

Tex at different stages exhibit distinct immunological characteristics and greatly influence the selection of immunotherapeutic strategies. Traditionally, the standard method of ICB therapy involves revitalizing the anti-tumour effector functions of CD8$^+$ T cells, including terminating or reversing tumour-induced CD8$^+$ T cell exhaustion.[84,85] However, the latest view suggests that interrupting the exhaustion process may impair the persistence of T cells in certain scenarios, and induction of T cell exhaustion should be considered first.[13] Combining the current strategies for intervening T cell exhaustion and inducing TLS, we propose a novel immunotherapy strategy (Fig. 3).

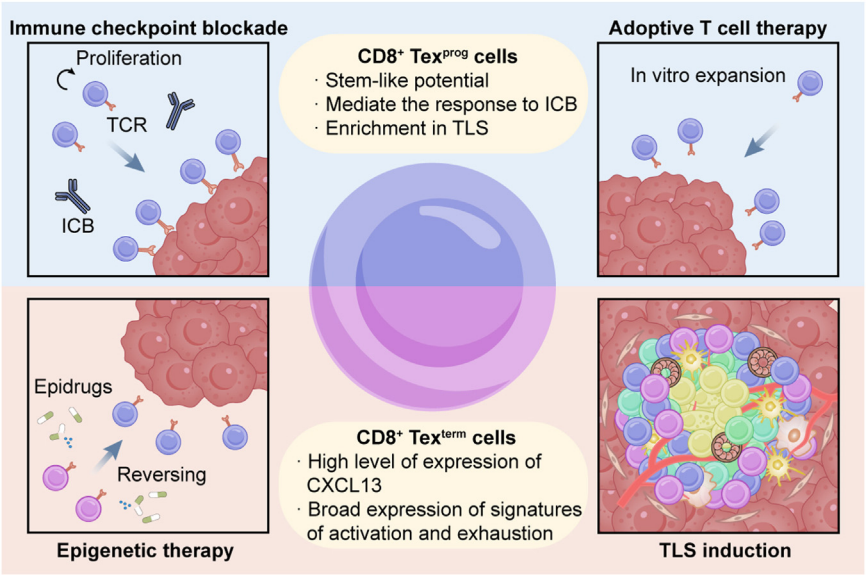

Fig. 3: **A novel immunotherapy strategy based on the crosstalk between T cell exhaustion and TLS formation**. In the TIME, Tex^{prog} retains the stem-like potential and is enriched in TLS to mediate the response to ICB treatment. Meanwhile, Tex^{term} broadly expresses the signatures of activation and exhaustion, and highly expresses CXCL13. Therefore, when the T cell subsets in the TIME are mainly Tex^{prog}, anti-tumour immune response can be enhanced by ICB treatment or ACT. When the T cell subsets in the TIME are mainly Tex^{term}, epigenetic therapy can be used to reverse the exhaustion state or induce intratumoral TLS.

Immunotherapy should be conducted when the function of the precursor-exhausted T cells is retained

The efficacy of ICB therapy depends on $CD8^+$ Tex^{prog}.[40] For example, PD-L1 upregulation should be a rationale for treatment only when pre-existing anti-tumour $CD8^+$ T cell responses are present, not in cases where other intrinsic tumour factors increase the expression of PD-L1.[13] Tex^{prog} retention of function and sustained infiltration are common in immunologically "hot" tumours. Furthermore, Tex^{prog} sourced from TDLNs are also of great importance as it relies on these peripherally mobilized and recruited T cells to complete "clonal replacement".[86,87] In brief, ICB treatment is effective only when Tex^{prog} cells are still present and retain their function. In addition, combination epigenetic therapies can be effective in combating ICB resistance.

TLS induction and ICB therapy should be given priority when facing strong tumour immunosuppression

Recent studies have shown that effective ICB treatment accelerates T cell exhaustion in some tumours, indicating that strong immunosuppression in the TIME can eventually lead to T cell dysfunction. T cell localized seclusion can provide some protection from tumour-mediated immunosuppression and an appropriate niche for anti-tumour immune responses.[49] Therefore, for ICB-unresponsive patients, inducing TLS should be considered to provide immunocytes with anti-tumour effects. Recent studies have reported advances in inducing TLS in vivo, including the use of important cytokines, modulators of key signalling pathways, tumour vaccines, chemotherapy, immunotherapy, and biomaterials, offering more options for patients with a low response rate to ICB therapy.[19,24,88,89]

Challenges

In recent years, significant advancements have been made in our understanding of T cell exhaustion and the development of TLS within tumours, yet numerous challenges remain. Single-cell omics technologies have revealed remarkable heterogeneity among tumour-infiltrating T cells, but their functional attributes are still not fully understood. Moreover, the formation and role of TLS in cancer are unclear, constraining their therapeutic potential. A major issue is the absence of stable TLS-bearing mouse tumour models for preclinical research, suitable for consistent use.

The different phenotypes of T cells that emerge during the process of T cell exhaustion have not been completely clarified

While the T cell exhaustion process has been relatively well-defined,[45,90] it is becoming increasingly clear that there is a significant heterogeneity in the mechanisms and manifestations of functional decline among T cells. Recent research has illuminated the existence of various subsets of terminally differentiated T cells that exhibit

distinct functional characteristics, such as T cell stress response state (Tstr),[91] tolerized CD8$^+$ T cells,[85] and bystander T cells.[44] These subsets are differentiated from the conventional Tex^{term} and Tex^{prog} and they have been found to be particularly enriched in tumours where there is a noted resistance to immunotherapy. The distinction between these "new" subsets and the conventional Tex^{term} is not merely academic but has profound implications for the design and implementation of immunotherapy. In the previous section, we proposed the potential role of CD8$^+$ Tex^{term} as a "call for help" within TIME. This view posits that CD8$^+$ Tex^{term} may signal for the recruitment or activation of other immune cells, potentially serving as a bridge in CI cycle. In future research, it will be necessary to further clarify exactly which subtype of CD8$^+$ Tex^{term} can participate in anti-tumour immunity, in order to design more targeted and effective immunotherapeutic strategies. Furthermore, considering that research on T cell exhaustion is largely based on animal models, it is essential to objectively and comprehensively evaluate the significance of these data while actively promoting clinical research.

The function of TLS in anti-tumour immunity still needs further investigation

The presence of TLS within tumours is not universally indicative of a favourable prognosis across all cancer types.[22,23,92–94] For instance, evidence from a study on pT1 bladder cancer revealed a correlation between TLS and unfavourable prognosis.[95] Even within the same disease, there is considerable heterogeneity in the clinical impact of TLS, which may be influenced by the complex molecular characteristics inherent in cancer.[24] In hepatocellular carcinoma (HCC), research has underscored that the occurrence of intratumoral TLS correlates with an increased risk of cancer recurrence and a diminished overall prognosis.[96] Moreover, in a study cohort of clear cell renal cell carcinoma (ccRCC), the association of immature TLS with a poorer prognosis has been observed. These findings introduce additional layers of complexity when predicting patient prognosis by detecting intratumoral TLS. Moreover, for patients with intratumoral TLS, receiving ICB therapy may not necessarily result in benefit.[88] The confluence of ICB therapy with TLS has been implicated in the emergence of immune-related adverse events (irAEs),[20,97] thus underscoring the necessity for a careful assessment of the risk-to-benefit ratio when employing such therapeutic interventions. In summary, a thorough understanding of TLS within the TIME is essential for refining prognostic assessments and crafting personalized treatment approaches for patients afflicted with cancer.

The deficiency of mouse models harbouring mature TLS constrains research into the TLS formation

The paucity of mature TLS in mouse models presents a significant challenge to the progression of TLS research. Inducing intratumoral TLS in mouse models has made some progress, including the use of transgenic mice or TLS-inducing therapies.[20,94,98] For instance, Johansson-Percival et al. induced intratumoral TLS in mouse model by targeting LIGHT and the vascular targeting peptide (VTP).[99] Additionally, Chaurio et al. constructed *CD4^{Cre}Satb1$^{f/f}$* mice and observed intratumoral TLS.[70] However, TLS are markedly rarer in transplanted tumour mouse models, and the maturity of TLS induced in mice is generally low, making them challenging to utilize for studies on mature TLS.[100] This emphasises the pivotal role of the TIME in the formation of TLS. Fridman et al. have proposed that the accelerated growth rate of transplanted tumours may impede the formation of TLS,[89] pointing to the dynamic interplay between tumour growth kinetics and the immune microenvironment. However, this view still lacks comprehensive empirical support, indicating a gap in our understanding of the mechanisms governing TLS formation in different tumour settings. It is also unknown what role T cell exhaustion plays in TLS formation. Moreover, the role of T cell exhaustion in the context of TLS formation remains an area of uncertainty. Research on this issue heavily relies on dynamic studies of the development process of TLS, yet conducting such studies using samples derived from cancer patients presents significant challenges. Given these considerations, there is an urgent need to identify mouse models containing TLS that are rapid, efficient, and highly reproducible.

Conclusions and outstanding questions

T cell exhaustion could be regarded as the trigger for TLS formation. Previous studies have focused on the expression of CXCL13 in Tex potentially associated with TLS formation.[49] Recent studies have not achieved good results in directly expressing CXCL13 in tumours to induce TLS, while over-expression of LTαβ or LIGHT has achieved amazing results.[17,101] In combination with our previous discussion, we believe that CXCL13$^+$ CD8$^+$ Tex cannot be simply regarded as the trigger for TLS formation. The T cell exhaustion process covers multiple aspects of the TIME, including activation of the CXCL13-CXCR5 axis, changes in TGF-β signalling, and IL-2 deprivation. Therefore, although it has been demonstrated that CXCL13$^+$ T cells are connected with TLS formation in tumours, T cell exhaustion should be regarded as the trigger for TLS formation.

Considering the complexity and heterogeneity of T cells, further exploration is needed to identify which T cells participated in the induction of TLS. A study in lung adenocarcinoma revealed an inverse correlation between the expression of CXCL13 in CD8$^+$ T cells and total cells,[58] suggesting that it is necessary to analyse specific cell subsets. Over the last ten years, the emergence of multi-dimensional single-cell technologies has offered unparalleled potential for dissecting complex

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed and Web of Science, and references from relevant articles using the search terms "tertiary lymphoid structure", "T cell exhaustion", "formation of tertiary lymphoid structure", "cancer immunotherapy", "exhausted T cell", "single cell RNA sequencing", and "cancer-immunity cycle". Due to the constraints on the number of references permitted for this review, a considerable portion of the primary literature could not be included. This Review primarily focused on literature published within the past 5 years.

lymphocytes in TIME.[40] In the future, we need higher resolution T cell atlases to guide immunotherapy. In addition, the spontaneous formation and induction mechanisms of TLS in tumours are still unclear, and it is important to monitor TLS and its corresponding components in real time in the future.

It is noteworthy that TLS has a certain special protective measure for internal T cells to prevent T cells from quickly losing effector function. Recent research found that the marker of $CD8^+$ T cell functional impairment can be established within hours after encountering tumour antigens, even earlier than the beginning of cell division.[102] The efficient protective mechanism of TLS for T cells can provide new schemes for improving the effect of ICB therapy and ACT in the future.

TLS participate in recruiting and modulating tumour-infiltrating T cells. The concept of the CI cycle encompasses seven sequential stages, beginning with the release of cancer cell antigens and culminating in the eradication of cancer cells.[30] A recent review expanded on the CI cycle, suggesting that T cells in the TIME can further proliferate and differentiate, directly cause tumour cell death, and form a localized "eddy" in the TIME.[31] In this review, TLS are considered to facilitate the interactions between tumour-infiltrating T cells and other immune cells.[31] Indeed, in conjunction with our discussion, the involvement of TLS in the CI cycle encompasses both regulating tumour-infiltrating T cells and facilitating T cell infiltration processes.

In conclusion, the overlap between T cell exhaustion and TLS formation in tumours may not be accidental. T cell exhaustion may be a "trigger" under more severe immunosuppression, activating a new round of immune response mediated by TLS.

Contributors

Wen-Ping Lin: Writing–original draft, Writing–review & editing, Visualization, Conceptualization. **Hao Li:** Writing–review & editing, Conceptualization, Funding acquisition. **Zhi-Jun Sun:** Writing–review & editing, Conceptualization, Funding acquisition. All authors have read and approved the final version of the manuscript.

Declaration of interests

The authors declare that they have no competing interest.

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