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Immunity

Review

Tertiary lymphoid structures and B cells: An intratumoral immunity cycle

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SUMMARY

The generation of anti-tumor immunity in the draining lymph nodes is known as the cancer immunity cycle. Accumulating evidence supports the occurrence of such a cycle at tumor sites in the context of chronic inflammation. Here, we review the role of tertiary lymphoid structures (TLS) in the generation of T and B cell immunities, focusing on the impact of B cells that undergo full maturation, resulting in the generation of plasma cells (PCs) producing high-affinity IgG and IgA antibodies. In this context, we propose that antibodies binding to tumor cells induce macrophage or natural killer (NK)-cell-dependent apoptosis. Subsequently, released antigen-antibody complexes are internalized and processed by dendritic cells (DCs), amplifying antigen presentation to T cells. Immune complexes may also be fixed by follicular DCs (FDCs) in TLS, thereby increasing memory B cell responses. This amplification loop creates an intra-tumoral immunity cycle, capable of increasing sensitivity of tumors to immunotherapy even in cancers with low mutational burden.

INTRODUCTION

Tertiary lymphoid structures (TLS) are lymphoid organs that develop in non-lymphoid tissues in response to antigen persistence in an inflamed microenvironment. They have been initially described in chronically infected organs,[1] in sites of inflammatory and auto-immune diseases[2,3] and in transplants subjected to chronic rejection.[4] In these situations, the presence of TLS is associated with exacerbated immune reactions to clear infections[5] or with aggravated tissue destruction in auto-immune diseases.[6] Tumors depict all features necessary for TLS formation: inflammation is chronic, and tumor cells express antigens resulting from aberrant gene expression, mutated genes, epigenetically modified self-molecules, re-expression of cancer-testis antigens, virally encoded proteins,[7] or bacterial proteins.[8] Indeed, TLS have been described in human cancers 15 years ago, in melanoma[9] and non-small cell lung cancer (NSCLC).[10] Although it is not deniable that lymph nodes are major sites of immunity generation, including in cancer,[11] recent data show that TLS can be sites of induction or reactivation of anti-tumor immunity.[12–14]

The term TLS has encompassed different lymphoid structures in the tumor microenvironment (TME), from T and B cell aggregates to organized structures with T cells surrounding B cells that form primary follicle (PFL) or secondary follicle (SFL) with a germinal center (GC).[12] Less organized structures likely being able to differentially impact tumor control have also been described, and it is necessary to reach a consensus to identify, characterize, and enumerate TLS. B cells are the dominant cell population inside TLS and, in contrast to T cells, are mostly confined to TLS in the TME.

In the present review, we propose a definition of TLS, a practical approach for their detection, and focus on the "*in situ*" generation of immunity toward tumors, addressing the questions of activation of naive or memory B cells, amplification and selection of B cell repertoires, maturation to plasma cells (PCs), antibodies produced by PCs, and the antigens they recognize. The impact of B cells and TLS on clinical outcome and patient's response to therapy is highlighted, underlying their use as biomarkers and tools for novel therapeutic approaches.

TLS AND OTHER IMMUNE AGGREGATES IN TUMORS

Organized structures that contain a B cell area adjacent to a T cell zone with mature dendritic cells (DCs) are collectively named TLS. Three easily identifiable classes of TLS have been characterized. Immature TLS (iTLS) are aggregates of T and B cells with few DCs. In such structures, there is no evidence of induction of efficient immune reactions, and they are rather associated with T-cell-exhausted, inflamed, and/or immunosuppressive TME, such as in hepatocellular carcinoma (HCC)[15] and luminal breast cancer (BC).[16] In luminal BC with iTLS, the exhausted-like T cells have an altered cytotoxic profile, and tumor cells express major histocompatibility complex class I (MHCI) molecules, suggesting a lack of immune evasion.[16] Mature TLS (mTLS) include a B cell area forming either a PFL or a GC-containing SFL.[17] In mTLS, mature DCs are in contact with T cells, and CD4+programmed death 1 (PD-1) +C-X-C chemokine receptor type 5 (CXCR5) + T follicular helper (Tfh) cells are in contact with B cells. The B cell zone contains a network of follicular DCs (FDCs) expressing CD21 in PFL as well as

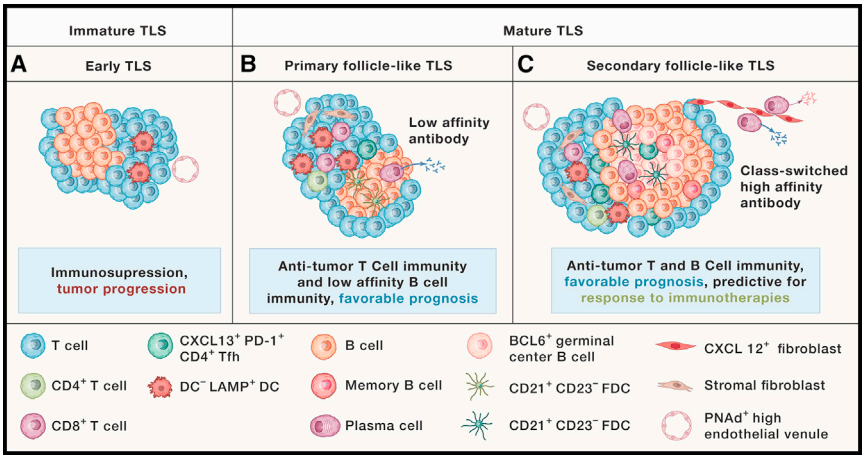

Figure 1. Intratumoral tertiary lymphoid structures

The figure illustrates the different types of tertiary lymphoid structures (TLS) found in tumors. TLS are defined as organized immune aggregates with distinct T and B cell zones, surrounded by peripheral node addressin (PNAd)-expressing high endothelial venules.

(A) Immature or early TLS contain mature dendritic cell lysosomal associated membrane glycoprotein (DC-LAMP)+ dendritic cells (DCs) in the T cell zone.

(B) Within mature TLS, primary follicle-like TLS have in addition T follicular helper (Tfh) cells and CD21+ follicular dendritic cells (FDCs) network allowing T cell immunity activation and low-affinity antibody production.

(C) Secondary follicle-like TLS are characterized by the presence of a germinal center (GC) with B cell lymphoma 6 (BCL6) positive GC B cells, CD21+CD23+ FDC allowing the production of memory B cells and high-affinity antibody secreting plasma cells. Boxes indicate their potential impact on cancer control and on clinical outcome. TCF1, T cell factor 1. Created with BioRender.com.

CD23 in SFL. Neighboring peripheral node addressin positive (PNAd+) high endothelial venules (HEVs), expression of B cell lymphoma 6 (BCL6), and production of the B cell/T cell attractant CXCL13 are also characteristics of mTLS[18,19](Figure 1).)

A practical algorithm that can be used in routine pathology laboratories to detect TLS and assess their maturity has recently been proposed.[20] In this study, tumor slides from 357 patients (211 carcinomas and 146 sarcomas) gathering 187 surgical specimens and 170 biopsies were analyzed by hematoxylin-eosin-saffron (HES) staining, immunohistochemistry (IHC), and multiplex immunofluorescence (mIF). Cellular aggregates that contain more than 50 cells on hematoxylin and eosin (H&E)/HES-stained slides and a characteristic filamentous network of FDC were classified as mTLS. Aggregates with no visible GC were further analyzed by IHC using anti-CD20 and -CD23 antibodies. Only TLS containing both CD20+ B cells and CD23+ FDCs characteristic of SFL were considered fully mature. If necessary, further distinction between iTLS, PFL-, or SFL-TLS may be realized by mIF staining including CD20, CD21, and CD23 enriched by CD3, CD4, CD8, PD-1, CXCR5, BCL6, and PNAd[19] (Figure 1). The use of artificial intelligence for TLS detection on H&E slides has gained attention[21,22] and may represent a future tool for pathology laboratories.

Although efficient in surgical specimens, TLS detection by H&E staining and IHC is less robust on small samples such as biopsies. It is therefore necessary to develop methods applicable to small tissue samples. In this regard, transcriptomic signatures have been proposed by several laboratories. Among those, several signatures can be used for different purposes. High expression of *CXCL13*, strongly associated with B cell chemotaxis, is easy to perform and robust[23] but may detect chemokine expression outside of TLS.[24] The 12chem signature contains genes encoding for myeloid, T cell attractants, and B cell attractants, showing a good correlation with the presence of TLS detected by IHC.[25] A Tfh cell signature, which includes CXCL13, has been proposed in BC.[26] A 29 gene signature, named TLS imprint signature, derived from spatial transcriptomic analyses, highly enriched in immunoglobulin (Ig) genes, genes expressed in PCs, with some T-cell-associated genes, defines mTLS in which full B cell maturation toward PCs occurs.[19] Other signatures, such as a T helper 1 (Th1)/B cell signature in gastric cancer[27] or a PC signature in ovarian cancer,[28] are linked to TLS-dependent functions but do not directly define TLS. Thus, as for the pathology algorithm, the choice of a transcriptomic signature depends on the question that is being addressed, CXCL13 being the simplest one, the 12chem signature including all types of TLS, and the 29 gene signature being specific for mTLS (Table 1).

Less organized immune structures containing T and B cells are seen in tumors, in addition to TLS (Table 2). Some exhibit pro-tumoral functions. Thus, intratumoral B-cell-rich immune hot spots containing T cells characterized by a low CD8/T regulatory (Treg) ratio are associated with unfavorable outcome in lung squamous cell carcinoma (LUSC).[30] Pancreatic ductal adenocarcinoma (PDAC) tumors present lymphoid aggregates of B cells and CD8+ T cells and small clusters of myelomonocytes and T cells in a context of high interleukin (IL)-10 expression. Short-term survivors exhibit decreased densities of CD8+ T cells in B and T cell aggregates as well as a higher level of co-localization of myelomonocytes and CD8+ T cells.[31] In early-stage lung cancers, clusters of CXCR5+ B cells close to CXCL13+ Tfh cells[32] and lung cancer activation modules (LCAMs) composed of IgG+ PCs close to PD-1+CXCL13+ activated T cells and secreted phosphoprotein 1 (SPP1)+ inflammatory monocyte-derived macrophages[33] have been described. It is not clear whether such B-cell-containing structures are TLS in formation or remain as so in the TME. The progressive increase in B cell content of the small clusters of Th cells (<16 cells)—called lymphonets—seen after tumor initiation in a mouse lung cancer model[34] suggest that TLS grow from a core of T cells and evolve to form more complex structures. The fact that small lymphonets containing T cell factor 1 (TCF1)+PD-1+ progenitor cells and cytotoxic cells progressively lose CD8+ T cells and acquire CD4+ Th upon size increase in early-stage human lung tumors supports this hypothesis and suggests that TCF1+PD-1+CD8+ T progenitor cells may play a role in TLS formation. The DC-CD4+CXCL13+ Th cell niches interacting with TCF1+PD-1+CD8+ T cells within cellular triads with DCs and populated by B cells but lacking effector T cells may also represent

Table 1. Gene signatures for the detection of tertiary lymphoid structures identified from transcriptomic analyses of human cancers

Signature name	Signature origin	TLS related functions	Genes	Reference
12chem signature	mRNA microarray analysis in primary colorectal cancer	involved in myeloid, B cell/T cell chemotaxis, cell adhesion, inflammation and cellular response to IFNγ, TNF; related to TLS at all maturity stages	CCL2, CCL3, CCL4, CCL5, CCL8, CCL18, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL13	Coppola et al.[25]
Tfh cell signature	qRT-PCR data analysis in breast cancer	involved in B cell and Tfh cell chemotaxis, cell recognition and T cell co-stimulation; detect GC+ mTLS	CXCL13, CD200, FBLN7, ICOS, SGPP2, SH2D1A, TIGIT, PDCD1	Gu-Trantien et al.[26]
Th1/B cell signature	mRNA microarray analysis in gastric cancer	involved in B cell/T cell proliferation, inflammatory response, T cell activation, Th cell differentiation and DC differentiation; not TLS specific	CD4, CCR5, CXCR3, CSF2, IGSF6, IL-2RA, CD38, CD40, CD5, MS4A1, SDC1, GFI1, IL-1R1, IL-1R2, IL-10, CCL20, TRAF6, STAT5A	Hennequin et al.[27]
PC signature	NanoString gene expression analysis in ovarian cancer	involved in adaptive immune response, response to TNF and B cell survival; not TLS specific	TNFRSF17, IGJ	Kroeger et al.[28]
CXCL13	bulk RNA-seq analysis in colorectal cancer and soft tissue sarcoma	involved in B cell and Tfh cell chemotaxis, germinal center formation, lymph node development and regulation of humoral immunity; highly expressed in TLS but not TLS specific	CXCL13	Petitprez et al.[23] and Becht et al.[29]
TLS imprint	spatial transcriptomic analysis comparing mature TLS zones vs. non-TLS zones in ccRCC samples	involved in immunoglobulin production, complement activation, and fibroblasts/B cell/T cell presence; highly xpressed in mTLS	IGHA1, IGHG1, IGHG2, IGHG3, IGHG4, IGHGP, IGHM, IGKC, IGLC1, IGLC2, IGLC3, JCHAIN, CD79A, FCRL5, MZB1, SSR4, XBP1, TRBC2, IL-7R, CXCL12, LUM, C1QA, C7, CD52, APOE, PTLP, PTGDS, PIM2, DERL3	Meylan et al.[19]

ccRCC, clear cell renal cell carcinoma; DC, dendritic cell; IFN, interferon; PC, plasma cell; Tfh, T follicular helper cell; Th, T helper cell; TLS, tertiary lymphoid structure; TNF, tumor necrosis factor.

TLS in formation.[35] Complementing these observations, TCF7+-PD-1+/− stem-like T cells enriched in metastatic melanoma tumors bearing TLS colocalize with B cells and show the highest densities around B cell aggregates and TLS, supporting *in situ* T cell priming mediated by TLS.[36] In addition, physical interactions between CD4+PD-1+CXCL13+ T cells and DC lysosomal associated membrane glycoprotein (DC-LAMP)+ mature regulatory DCs (mregDCs) were found inside TLS in BC and melanoma. These T cells exhibited high clonality suggesting education toward tumor antigens.[37] The precise location and interacting partners of the TCF1/*TCF7*+ T progenitor cells present during the different maturation steps of TLS need further investigation as does the respective contributions of immune aggregates, TLS, and perivascular niches to the formation of differentiated intratumoral T cell progeny.

In contrast, structures lacking, or with few, B cells, such as the stem immunity hubs reported in NSCLC, characterized by *TCF7*+CD8+ T cells and enriched for the interferon (IFN)γ gene set signature seem to be distinct from TLS[39] as are the immune hubs composed of CXCL13+ T cells, and IFNγ+ T cells close to myeloid and tumor cells expressing the T-cell-attracting chemokines CXCL10/CXCL11+ present in mismatch repair-deficient (MMR-D) colorectal cancer (CRC)[38] and the antigen-presenting cell (APC) niches containing TCF1+CD8+ T cells and MHCII APCs found in urologic tumors.[40] Additionally, TCF1+PD-1+CD8+ T cells found in perivascular niches in mouse

Table 2. Cellular composition of immune aggregates

Immune aggregates	Cancer types	T cells	B cells	PC	Myeloid cells	Pronostic value	Effect of immunotherapy	Ref
Immune hot spots	NSCLC	CD8+ T cells, regulatory T cells	B cells	–	–	poor outcome	–	Zhang et al.[30]
LCAM	NSCLC	PD-1+ CXCL13+ T cells	–	CD138+ PC	SPP1+ mono-macrophages	–	PFS increase in LCAM high patients receiving anti-PD-L1	Leader et al.[33]
T and B cells	NSCLC	CXCL13+ T cells	CXCR5+ B cells	–	–	–	–	Hao et al.[32]
Immune clusters	PDAC	IL-10+CD4+ T cells	–	–	IL-10+ myelomoncytes	spatial proximity in long-term survival patients	–	Mi et al.[31]
		GZMB+CD8+ T cells	–	–	IL-10+ myelomonocytes	spatial proximity in short-term survival patients	–	
Lymphoid aggregates		CD8+ T cells	B cells	–	–	low CD8+ T density in CD8+ T and B cells aggregates in short-term survival patients	–	
Lymphonets	NSCLC early stage	TCF1+PD-1+CD8+ T progenitor cells, cytotoxic cells, CD4+ Th cells when large	B cells when large	–	–	–	–	Gaglia et al.[34]
Immune hubs	MMR-D CRC	CXCL13+ T cells, IFNγ+ T cells	–	–	CXCR3 ligand+ myeloïd cells	–	–	Pelka et al.[38]
Stem immunity hubs	NSCLC	TCF7+CD8+ T cells	–	–	*CXCL10*+ macrophages adjacent to CD8+ T cells; *CCL19*+ mregDC adjacent to CD4+ T cells (including Treg)	–	PFS and OS increase in patients responding to anti-PD-1/PD-L1	Chen et al.[39]
APC niche	RCC, bladder, prostate	TCF1+CD8+ T cells	–	–	MHCII+ APC	low density in patients with low progression-free survival	–	Jansen et al.[40]
Immune triad	HCC	CD4+CXCL13+ Th cells; TCF1+PD-1+CD8+ T cells	B cells	–	mregDC	–	increased in responders to anti-PD-1	Magen et al.[35]
Immune niches[a]	melanoma	TCF1+PD-1+CD8+ T cells producing cytotoxic effector T cells	B cells+/−	–	–	*TCF7*/*PCDC-1* signature correlates with improved survival	increased in patients receiving anti-PD-1 or anti-CTLA-4	Siddiqui et al.[41]

GZMB, granzyme B; IFN, interferon; LCAM, lung cancer activation module; LUSC, lung squamous cell carcinoma; MHC, major histocompatibility complex; MMR-D CRC, mismatch repair deficient colorectal cancer; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; RCC, renal cell carcinoma; SPP1, secreted phosphoprotein 1; TCF1, T cell factor 1.
[a]Located in TLS and in perivascular niches outside TLS.

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models[42,43] and human melanoma[41] are associated with tumor control and response to immunotherapy. In contrast, "stress response T cells" (Tstr), defined by the expression of stress-related heat shock genes, notably HSPA1A and HSPA1B,[44] are predominantly observed in lymphoid aggregates in numerous cancers types, associated with resistance to immune checkpoint blockade.

Altogether, these results illustrate the tumor-cell-dependent and functionally diverse immune aggregates that can be present in the TME (Table 2). They suggest that intratumoral immune aggregates can act as niches to support the differentiation of TCF1/*TCF7*+ T cells into effector T cells. More data are required to establish if these different structures relate between themselves or with TLS.

Although TLS[45] and immune niches[36] appear to be sites for generating or re-activating anti-tumor T cell immunity, it may also occur in draining lymph nodes,[11,46] with educated T cells penetrating the tumors through HEV.[47]

In addition to their maturity, another parameter that is essential for the impact of TLS on tumor control is their location in the TME. Thus, TLS present in the tumor core or the invasive margin, close to tumor cells, may be sites of the generation of immune responses to tumor-associated antigens and are usually associated with favorable clinical outcome in many cancers. In contrast, TLS located in inflamed tissues, at a distance from tumor beds, may represent sites of immunity against self-antigens that are not over-expressed in tumor cells and are not directly involved in cancer control.[48–50] The question of the location of TLS is also of paramount importance when analyzing metastases in lymph nodes; only intra-tumoral and invasive margin TLS should be taken into account, lymphoid structures at distance being likely non-tumor-associated secondary lymphoid organs in reaction to a variety of antigens present in reactive lymph nodes.

MODULATION OF THE B CELL REPERTOIRE AND ISOTYPE SWITCHING IN TLS-CONTAINING TUMORS

As in secondary lymphoid organs, GCs in TLS may be sites of selection, amplification, and affinity maturation of B cell repertoires as well as isotypic switching, resulting in PC generation. Presence of IgG and IgA+ PCs in tumors from a large array of cancer types, particularly in ovarian cancer[51–53] or soft tissue sarcoma (STS),[54] together with the concomitant presence of antibodies bound to tumor cells[13,54–57] support this hypothesis.

Direct evidence for the generation of full B cell responses comes from studies analyzing B cell repertoires in tumors. In clear cell renal cell carcinoma (ccRCC), accompanying the generation of plasmablasts and PCs in the GC of TLS, Meylan et al.[19] found much higher numbers of Ig heavy (IgH)-chain and Ig light (IgL)-chain clonotypes in tumors with a high TLS imprint signature expression (defined as being over the median of the analyzed cohort) compared with tumors with a low TLS imprint signature expression. IgH clonotypes represented more than 30 times were dominant, suggesting selection and amplification of B cell clones. Spatial transcriptomics revealed a high range of mutation counts of IgL clonotypes inside TLS, whereas only highly mutated clonotypes were detected outside TLS in the same tumors. This suggests that hypermutation is occurring inside TLS and that only PCs that have undergone affinity maturation travel into the tumor beds. Indeed, the same IgH clones were located both inside TLS and at a distance from the tumor beds.[19] Thus, TLS appear to be the sites of the generation of B cell immunity from naive or memory B cells to PC eventually yielding to antibody production, mostly IgG and IgA, toward tumor cells.[19] In high-serious-grade ovarian cancer (HSGOC), Mazor et al.[55] reported that accompanying the presence of IgG antibodies bound to tumor cells, TLS and IgG1 antibody-secreting PCs are present in the stroma surrounding tumor nets. Single-cell sequencing of heavy and light chains mRNAs in B cells sorted from fresh tumors revealed clonotype expansion, somatic hypermutation, and clonal diversification by progressive somatic hypermutations on both Ig chains. Also, in HSGOC, Biswas et al.[53] reported high numbers of IgA- and IgG1-secreting plasmablasts and PCs associated with improved patient survival and coating of tumor cells, essentially with IgA antibodies. In a mouse model of ovarian cancer, intra-tumoral isotype switching of B cells is induced by Tfh cells in TLS.[58]

These complementary approaches in different cancers converge to support that intratumoral TLS are sites for anti-tumor antibody production: (1) mature SFL-TLS contain a GC wherein selection, amplification, affinity maturation, and isotype switching occur, leading to the generation of PCs, mostly producing IgG and IgA; (2) polyclonal activation of intratumoral B cells from TLS-rich lung tumors induces the production of IgG and IgA antibodies to cancer-associated antigens[59]; (3) high percentages of IgG-coated tumor sarcoma cells[54] and ccRCC cells[19] are found in tumors with TLS and high densities of IgG+ PCs; and (4) antibodies produced based on single-cell reconstitution of heavy- and light-chain repertoires of intra-tumoral B cells in HSGOC react with ovarian cancer cell lines[55] as do antibodies present in ascitic fluids of ovarian cancer patients.[53] Without excluding the possibility that anti-tumoral antibody responses could be generated in peripheral lymphoid organs, the above findings militate in favor of *in situ* antibody production and raise the questions of their specificities and functions.

NATURE, SPECIFICITIES, AND FUNCTIONS OF *IN-SITU*-PRODUCED ANTIBODIES TO TUMOR CELLS

The presence of antibody deposits on tumor cells seems to be a general phenomenon. Analyzing 35 tumor types, Mazor et al.[55] reported that with the exception of fibrosarcoma, a large array of solid tumors stain with anti-IgG antibodies, revealing IgG bound to tumor cells. Although all cancer types exhibit a subset of tumor cells coated with IgG, their percentages vary according to the tumor type from a median over 50% in RCC, liposarcoma, urothelial carcinoma, and testicular cancers to less than 20% in other cancer types including lung carcinoma, melanoma, ovarian cancer, uterine and cervix carcinomas, BC, HCC, CRC, pancreatic cancer, and glioblastoma.[55] In HSGOC, both IgA and IgG label tumor cells.[53] In ccRCC, there is a median of 70% of IgG-coated tumor cells and a lower content of IgA-coated tumor cells.[19] As a general rule in these studies, the level of IgG and IgA antibodies bound to tumor cells associated with the presence of intratumoral TLS where isotype switching took place.

The quest for antigens is a matter of intense research since it may lead to the identification of novel targets for immunotherapies. Cancer-testis antigens, mutated p53 and BRCA2 proteins, or lineage-specific antigens in lung cancer, BC, or ovarian

cancer recognized by IgG and IgA antibodies are mostly intracellular (reviewed in Laumont et al.[56] and Germain et al.[59]). In head and neck squamous cell carcinoma (HNSCC), human papillomavirus (HPV)+ tumors, IgG, and IgA antibodies recognize intra-nuclear E6 and E7 HPV proteins,[60] and in lung cancer, IgG antibodies specific to the re-expressed envelope antigen of endogenous retroviruses (ERVs) are produced in TLS.[61] Several reports also identified antibodies to molecules dysregulated in cancer such as membranous mucin 1 (MUC1) in BC[62] or tetraspanin N7 in ovarian cancer[53] as well as antibodies targeting intracellular proteins.[56,63] A large number of reactivities of *in-situ*-produced antibodies are with intracellular or membranous self-antigens in lung, ovarian, or BCs shared by malignant and normal cells.[56] Thus, very few tumor-specific targets have been identified raising the question of whether these antibodies exert only anti-tumoral or also auto-immune activities.[57] The fact that many antigens are intracellular supports the hypothesis that antibody responses are induced to antigens released by dying tumor cells, which may be particularly occurring in hypoxic tumors such as RCC. Cell death can also be induced by therapies, including radio-, chemo-, or immune-therapy.[64] Thus, antigens that are usually not accessible—sometimes tumor-specific, such as virally encoded onco-proteins or cancer-testis antigens as well as intracellular self-antigens—are not controlled by central tolerance and may thus not be subjected to immunoediting, and they can become immunogenic and induce antibody responses.

PROGNOSTIC IMPACT OF TLS AND B CELLS

As discussed above, the presence of TLS in the TME, in close proximity to tumor beds, correlates with favorable patient prognosis[13] as evidenced in all cancers in both primary and metastatic sites.[65] In contrast, TLS are not associated with longer patient survival if they are located at a distance, outside the invasive margin, of tumor nests.[48,49] In addition, the question of whether there is a quantitative relationship between the number of TLS, or the expression level of TLS signatures, and clinical impact or if there is a tumor-dependent threshold of the number of TLS required for favorable clinical impact remains open. What is established is that the presence of intra-tumoral TLS influences the clinical impact of different cell types that may have different functionalities in tumors with or without TLS.

Considering T cells, there is a general consensus that high T cell densities in the tumor center and its invasive margin correlate with favorable prognosis.[66] Among T cell subsets, memory T cells, which represent the vast majority of infiltrating T cells,[67] and particularly memory CD8+ T cells are major players in anti-tumor immunity,[68] even if most of them may be anti-viral.[69] In tumors containing TLS, the favorable clinical impact of CD8+ T cells appears to depend on TLS.[14,45] Presence of Treg cells in TLS counteracts the positive clinical impact of CD8+ T cells in NSCLC,[70] CRC,[71] or STS,[54] as well as in a mouse model of lung cancer.[72] Among CD4+ T cells, CXCL13-producing Tfh cells represent another T cell subset principally located in TLS. An eight-gene Tfh signature predicted survival or preoperative response to chemotherapy in BC.[26] In a mouse model of CRC, microbiota-specific Tfh cells are induced in tumor-adjacent TLS potentially leading to CD4+ T and B cell activation and resulting in tumor control.[73] In HNSCC, single-cell transcriptomic analyses of HPV+ and HPV− tumors followed by the analyses of ligand-receptor interactions revealed clusters of B cells interacting with CD4+ T cells inside TLS in both HPV− and HPV+ tumors and with B cells and Tfh in HPV+ tumors supporting a B cell activation process linked to tumor control in HNSCC.[74]

What is the impact of B cells on disease prognosis? In humans, there is clear evidence of higher B cell densities in tumors with TLS than in tumors without TLS or in the corresponding normal tissues. It is particularly the case for memory B cells and PCs, the latter being virtually undetectable in most non-lymphoid normal tissues.[56] Strikingly, in most cancers, B cell, PC, and IgG enrichment scores in the tumor correlate with good prognosis, whereas IgA score was found associated with poor prognosis in NSCLC, BC, melanoma,[56] and HCC,[75] but with good prognosis in ovarian cancer.[53]

Since all B cell subsets are found in tumors,[13] it raises the question of their different impacts on patient prognosis. However, many studies have been conducted, identifying B cells with the pan-B cell marker CD20, which is expressed on all B cell subsets, except long-lived PCs. In contrast to several mouse models in which B cells are associated with pro-tumoral activities,[76–80] the density of B cells in the TME correlates with favorable prognosis in a large array of tumor types in human studies.[13,56,81] This striking difference between mice and humans may be due to different factors among which is the lack of TLS formation in most murine models (reviewed in Fridman et al.[13]). Since, in human tumors, the intra-tumoral density of B cells is highly dependent on the presence of TLS, they may be educated *in situ* in a different manner than that in TLS-lacking mouse tumors and accomplish anti-tumoral effector functions. Data are heterogeneous, concerning the impact of the different B cell subsets on clinical outcome. In the different studies, B cell subsets have been characterized and quantified either by using transcriptomic signatures derived from single-cell RNA-seq or by immuno-imaging with antibodies to specific markers or both methods.[13] With the exception of B regulatory (Breg) cells, all B cell subsets have generally been associated with favorable clinical outcome, including naive B cells, switched memory B cells, GC B cells, plasmablasts, and PCs.[13,56] The case of Breg cells is still a matter of debate, due in great part to the lack of a consensus markers to identify this subset.[82] However, using IL-10 or transforming growth factor β (TGF-β) production as a proxy for Breg cells, correlation with poor prognosis has been reported in several cancers.[83–86] In PDAC, the proportion of IL-35-producing B cells inversely correlate with that of PCs, suggesting that IL-35 contributes to intratumoral B cell dysfunction and subsequent inhibition of PCs generation.[87] In terms of mechanism, the stimulator of IFN genes (STINGs) seems to play a major role in the induction of these immunoregulatory B cells since the administration of STING agonists results in an expansion of human and mouse IL-35+ Breg cells in IRF3-dependent but IFN-independent manner in pancreatic cancer.[88] The impact of these cells was established in a mouse model in which IL-35 blockade or genetic ablation reduced tumor growth.[88] Altogether, these studies indicate that immunoregulatory B cells can be expanded in different cancer types—further research being necessary to better characterize their phenotypes and functions through induction of Treg cells or direct inhibition of effector CD8 T cells or PC generation (reviewed in Michaud et al.[82]).

In humans, it is clear that the density, fate, and functions of B cells are highly dependent on the presence and maturity of TLS. Thus, tumors lacking TLS have generally a low level of B cell infiltration that may occur directly through conventional CD31/CD34+ blood vessels.[13] There is no evidence that such B cells, when present, are educated toward tumor-associated antigens and exert tumor control. In tumors with TLS, B cells enter into these lymphoid structures through PNAd+ HEV. Both naive and memory B cells traffic this way,[12] and TLS are the only sites in tumors where naive B cells can be found.[89] In iTLS that are associated with an immunosuppressed IL-10 and TGF-β-rich milieu in early stage HCC nodules,[15] activation of B cells may be diverted toward the generation of Breg cells, whose presence correlates with deleterious clinical impact in several cancers.[82] In mTLS, anti-tumor B cell immunity can be generated either by activation of naive B cells or reactivation of memory B cells that had been activated in draining lymph nodes and have entered TLS through the HEV. It may occur inside the T cell zone and GC upon antigen presentation by immune complexes bound to FDCs and signals delivered by Tfh cells located at the periphery of the GC. In NSCLC, it has been proposed that naive B cells are activated and undergo full maturation toward PCs.[59] In RCC, spatial transcriptomic analyses revealed that the memory B cells are a hallmark of mTLS and concentrate in GC where naive B cells are rather scarce,[19] pleading for a reactivation *in situ*. However, high numbers of memory B cells may also reflect *in situ* maturation of naive B cells, leaving the question of their priming site still open. Other B cell subsets localized in TLS such as GC B cells and IgM-producing plasmablasts present only in TLS GC. PCs are generated in TLS and travel into tumor nests along CXCL12+ fibroblastic conducts.[19] The impact of PCs on clinical outcome has raised huge interest since they produce antibodies that could be used as therapeutic tools. Using public databases, Laumont et al.[56] reported that expression of PC transcriptomic signature was higher in tumoral vs. normal tissues and that PC and IgG high transcriptomic scores correlate with favorable prognosis in the vast majority of cancer types. Other studies reported that high densities of PCs detected by IHC correlated with good prognosis in ovarian,[28,51] head and neck,[90] or prostate[91] cancers.

Finally, tumor-cell-bound antibodies also influence clinical outcome. In HSGOC, patients with IgG+-tumor cells have a longer disease-free survival (DSS) and overall survival (OS) than patients with no IgG on their tumor cells.[55] Also in HSGOC, Biswas et al. found a positive correlation between the level of IgG and IgA antibodies on tumor cells and clinical outcome.[53] In lung cancer, antibodies to endogenous retrovirus (ERV) membrane glycoproteins, mostly of IgG and IgA isotypes, generated in TLS, are amplified in immune checkpoint inhibitor (ICI)-treated patients and high levels of serum anti-ERVK-7 correlate with longer survival in patients with lung adenocarcinoma.[61]

TLS AND B CELLS PREDICT THERAPEUTIC RESPONSES AND LONGER SURVIVAL IN PATIENTS TREATED WITH IMMUNOTHERAPY

Effective immunotherapies aim to reinvigorate anti-tumor T cell immunity at tumor sites and are commonly targeted to T cells as exemplified by the broadly approved ICIs blocking cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)[92] or the PD-1-programmed death-ligand 1 (PD-L1) axis.[93] It is therefore not surprising that many reports underline the necessity of an abundant intratumoral T cell infiltrate to sustain therapeutic responses to ICI.[68,94] As hallmarks of the importance of the T cell compartment are the group of tumors with microsatellite instability (MSI). In such tumors, deficiency of mismatched DNA repair enzymes results in the generation of high numbers of tumor mutations and subsequent strong infiltration of CD8+ T cells in the TME. Other effector cells such as Tγδ cells may also play a role in MHCI-negative MMR-D colon cancers.[95] Patients with MSI tumors have a high response rate (>50%), a long progression-free survival (PFS), and OS when treated with pembrolizumab, an antibody against PD-1.[96,97] These findings strongly support that CD8+ T cells recognizing mutated tumor-associated peptides presented by MHCI molecules are responsible for tumor control. However, in patients with MSI CRC treated with ICI in a neo-adjuvant setting, who all responded to the treatment, there is an increase not only in T cells but also in macrophages and TLS after treatment. Moreover, the most increased transcriptomic signatures upon ICI therapy were, in addition to the expected IFNγ signature, CXCL13- and TLS-associated genes.[98] In patients with rectal cancer treated with neo-adjuvant dorstalumab, an anti-PD-1 antibody, which yielded over 80% therapeutic responses, B cells, and TLS also increase during treatment.[99] These findings illustrate the fact that even in cancers that are considered as exemplary situations of T cell control, other players of the TME may be actors of the therapeutic responses such as TLS, which are sites of generation of both B and T cell immunities, and macrophages that contribute to effector activities against tumor cells. Patients with bladder cancer presenting high-risk features were treated in a neo-adjuvant setting with a combination of anti-PD-L1 (durvalumab) and anti-CTLA-4 (tremelimumab) antibodies. Complete pathological responses (pCRs) were observed at the time of surgery in 9 of the 24 patients. Analysis of pre-treatment samples evidenced a higher number of TLS in responding patients as well as a longer OS and recurrence-free survival (RFS) in patients with TLS numbers above the median. The other elements in the TME that predict responses were high densities of B cells, CD4+ T cells, and CD8+ T cells, whereas PD-L1 expression on tumor and infiltrating cells had no significant impact on response.[100] In another trial conducted in patients with locoregionally advanced urothelial cancer pre-operatively treated with a combination of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1), baseline level of CD8+ T cells, or effector T cell signatures did not predict response, whereas induction of TLS signatures was observed in responding patients.[101] In patients with NSCLC treated with neo-adjuvant anti-PD-1, the presence of post-treatment TLS and PCs were detected only in responding patients correlating with high lymphocyte infiltration[102] (Table 3). In other neo-adjuvant trials in NSCLC,[103] urothelial cancer,[104] HNSCC,[105] and T cell signatures were increased in responding tumors, but the presence of TLS and B cells was not reported.

In early-stage lung cancer, high baseline LCAM scores correlated with enhanced NSCLC response to immunotherapy.[33] The relative importance of LCAM scores and TLS content needs further investigation to better elucidate the specific impact of LCAM on the immunotherapy response.

Table 3. Impact of TLS and B cells on responses to immunotherapies

	Cancer types	Treated by	Cell type predictive for response	Modifications in responding patients	Ref
Neoadjuvant ICI					
	MMRd CRC	αPD-1 + αCTLA-4		CD3+ T , Macrophages increased IFNγ, CXCL13, TLS signatures increased	98
	Urothelial carcinoma	αPD-L1 + αCTLA-4	TLS, B cells, CD4+ T and CD8+ T cells signatures		100
		αPD-1 + αCTLA-4	No	TLS increased	101
	NSCLC	αPD-1		TLS and PC presence in regressing tumors	102
Metastatic cancers					
	NSCLC	αPD-1	LCAM		31
	Melanoma	αPD-1 /αPD-1 + αCTLA-4	TLS, CD20+ B cells, IGH & IGL clonotypes	B cells and TLS increased	111
	Melanoma	αPD-1 /αCTLA-4	TLS signature in CD8+ T and CD20+ B cells high tumors		104
	ccRCC	αPD-1 /αPD-1 + αCTLA-4	B cells signature		112
	ccRCC	αPD-1 /αPD-1 + αCTLA-4	TLS, IgG antibodies on tumor cells		19
	ccRCC	αPD-1 + αCTLA-4	B cell associated signature		113
	STS	αPD-1	immune high group characterized by TLS presence		23
	STS selected for presence of TLS	αPD-1	activated DC and macrophages, Ig gene signatures		54
	NSCLC, STS, urothelial, CRC, RCC, BC and others	αPD-1 /αPD-L1	mature TLS		18
	CIN high grade	E6/E7 HPV 16 vaccine	TLS induction		117
	PDAC	GVAX	TLS induction		118

BC, breast cancer; ccRCC, clear cell renal cell carcinoma; CIN, cervical intraepithelial neoplasias; CRC, colorectal carcinoma; CTLA-4, cytotoxic T-lymphocyte-associated protein-4; DC, dendritic cell; GVAX, granulocyte-macrophage colony-stimulating factor gene-transfected tumor cell vaccine; HPV16, human papillomavirus serotype 16; ICI, immune checkpoint inhibitor; IFN, interferon; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; LCAM, lung cancer activation module; MMR-D CRC, mismatch repair deficient colorectal carcinoma; NSCLC, non-small cell lung cancer; PC, plasma cell; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1; PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; STS, soft-tissue sarcomas; TLS, tertiary lymphoid structure.

These data raise the question of the impact of TLS and B cells, alone or in conjunction with CD8+ T cells, in metastatic patients when tumors have undergone extensive immuno-editing.[107–109] A study that combined whole-exome and transcriptomic data from >1,000 ICI-treated patients across seven tumor types and single-cell RNA-seq from T cells demonstrated that CXCL13 is expressed in the T cells reactive to a clonal neoantigen and also in responders to ICI.[110] In lung cancer both in human and in an immunogenic mouse model, high B cell signature expression, the presence of TLS with a GC, and the production of anti-tumor antibodies reacting with endogenous retroviruses correlated with longer survival. Indeed, transfer of serum from mice containing anti-tumor antibodies increased recipient survival requiring the presence of natural killer (NK) cells.[61] In the mouse model, longer survival of mice treated with ICI, particularly with anti-PD-L1 antibodies, required the presence of B cells and CXCL13, intranasal injection of which was synergistic with PD-L1 blockade.[61] In metastatic melanoma and RCC, patients treated either with anti-PD-1 antibodies (nivolumab) alone or a combination of anti-PD-1 and CTLA-4 (ipilimumab) antibodies, analysis of pre-treatment resected tumors revealed that TLS and B cell densities predicted responses and that they increased upon treatment in responding patients as well as expanded IgH and IgL clonotypes,[111] suggesting that they could also be used to monitor efficient immunotherapies. Analyses in melanoma patients also revealed that in pre-treatment tumors, the densities of T cells, NK cells, and myeloid cells are not associated with response.[111] In another study conducted in metastatic melanoma patients, although densities of both CD8+ T cells and B cells in the TME were associated with longer OS, B cells seemed to be dominant since patients with tumors presenting with high CD8+ T cell density, but low B cell density had a shorter survival. In addition, analyzing several cohorts of melanoma, the authors reported that patients presenting with high TLS transcriptomic signature expression had a longer OS in ICI-treated patients even in tumors with high CD8 and B cell signatures. Finally, TLS density was independent of the tumor mutational burden (TMB).[106] Using MCP-counter,[29] a transcriptomic immune classification of over 800 STS tumors from TCGA and other cohorts identified an immune high group with high signatures expression of all immune cells in the TME, independent of the STS histotype. This group was associated with longer OS. Among the different immune cell signatures, only the B cell signature independently of the T cell signatures, correlated with longer OS.[23] Using transcriptomic gene signature and IHC, the authors showed that TLS presence was a hallmark of the immune high group and was associated with high densities of T and B cells in the TME. In patients with metastatic STS treated with the anti-PD-1 antibody pembrolizumab, the response rate was 50% in the immune high group compared with 0% in the immune low group and the immune high group had longest PFS.[23] In metastatic ccRCC patients treated with nivolumab (anti-PD-1 antibody) alone or in combination with ipilimumab (anti-CTLA-4 antibody), the presence of TLS correlated with the presence of IgG antibodies on tumor cells that was associated with high response rates and longer PFS.[19] The expression of a TLS and B cell-associated transcriptomic signature including CXCL13 and Ig genes was associated with response to ICI and not to anti-angiogenic tyrosine kinase inhibitor (TKI).[112] A transcriptomic immune classification of the reputed non immunogenic glioblastoma tumors identified an immune group characterized by the presence of TLS and high expression of Ig genes; this group strongly associates with response to ICI, particularly in patients treated with neo-adjuvant immunotherapy[113] (Table 3).

Together with the data from the neo-adjuvant trials, these studies in metastatic cancers bring to light several major findings as follows: (1) TLS and B cells predict therapeutic responses to ICI, in conjunction or not with CD8+ T cells and appear to be important for the clinical outcome of patients treated with ICI, (2) location of TLS in the tumor core or at its close invasive margin is required to predict favorable clinical outcome[23] particularly when metastases in lymph nodes are analyzed,[106] (3) TLS and B cell signatures are independent of the TMB and predict therapeutic responses to ICI both in highly mutated tumors such as melanoma[106,111] or poorly mutated tumors such as STS[23] or RCC,[19,111] and (4) finally, most TLS depicted in these studies are mature thus supporting the hypothesis that mature, rather than iTLS, are associated with responses to immunotherapy.[114] Indeed, retrospective analysis of 540 patients from 3 independent cohorts, containing a large array of cancer types (NSCLC, STS, head and neck cancers, RCC, bladder cancer, CRC, etc.) treated with PD-1/PD-L1 blockers, revealed that the presence of mature and not immature, TLS correlates with higher response rates and longer PFS and OS compared with tumors without TLS. The impact of mTLS is independent of the expression of PD-L1 on tumor and infiltrating cells and significant even in patients with high (above the median) or low CD8+ T cell infiltrate in the TME.[18] The expression of pre-treatment PC-associated signatures is predictive of therapeutic responses to ICI in NSCLC.[115] Thus, the positive impact of mTLS and B cells in ICI-treated patients is a general phenomenon occurring in many, if not all, cancer types.

Based on these findings, a prospective trial was launched treating 30 metastatic STS patients selected for the presence of TLS in their tumor with pembrolizumab (anti-PD-1 antibody) and low-dose cyclophosphamide. The trial yielded 30% partial responses (PRs), 33% stable diseases (SDs), and 33% progressors[54] compared with 2.4% PRs in a similar cohort of non-selected patients subject to the same treatment.[116] The non-progression rate at 6 months was 40% in the TLS-selected cohort and only 4.9% in the non-selected cohort, supporting the use of TLS for selection of patients to be treated with ICI. Pre-treatment immunological analyses of the tumors, which all contained TLS, revealed that responding patients had a high expression of Ig genes and a high density of IgG+ PCs in the TME, some showing IgG labeling on their tumor cells. In addition to PCs, high densities of activated DCs and activated macrophages in the tumor beds correlated with longer PFS and OS, whereas high density of Treg inside TLS were found in non-responding patients and correlated with short PFS and OS.[54] Altogether, these data identify TLS and B cells as pivotal elements for response to ICI.

TLS were also associated with responses to other immunotherapies. After vaccination of patients presenting with high-grade cervical intra-epithelial neoplasia, active TLS were induced in the target lesions supporting a tissue-localized immune response in patients systemically treated with the anti-E6/E7 antigens of HPV16 DNA vaccine.[117] Similar results were

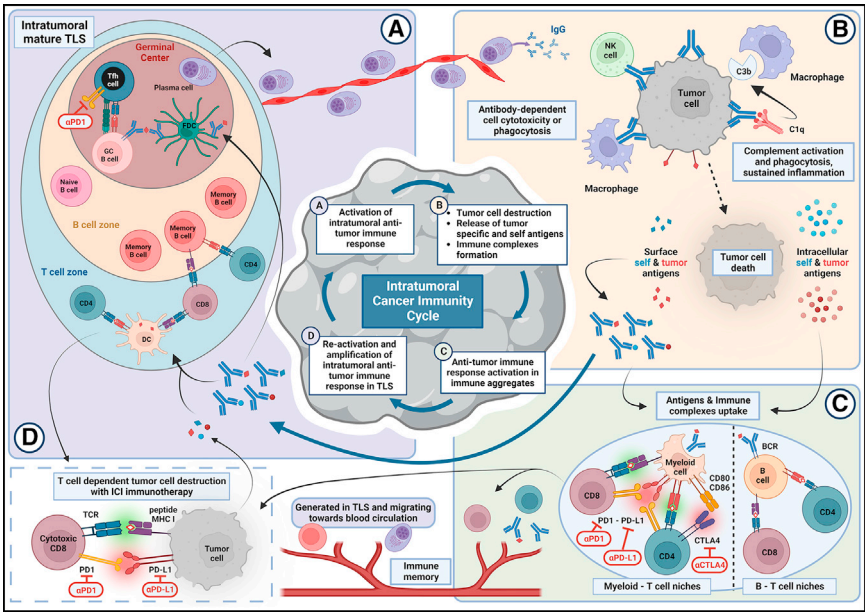

Figure 2. A tertiary lymphoid structure-dependent intratumoral cancer immunity cycle promotes response to immune checkpoint inhibitors

(A) Activation of intratumoral anti-tumor immune response in TLS: in the T cell zone, dendritic cells (DCs) capture antigens, and present the processed peptides on major histocompatibility complex (MHC) I and II molecules to CD8+ and CD4+ T cells, respectively, leading to the formation of effector anti-tumor cytotoxic T cells and T helper cells. The B cell follicle includes memory and naive B cells, activated by native antigens and T follicular helper (Tfh) cells. In germinal center (GC)-containing TLS, activated B cells mature antibody affinity by interacting with Tfh and follicular dendritic cells (FDCs)-presenting antigens in the form of immune complexes, leading to the generation of high-affinity class-switched memory B cells and plasma cells. B cells can also act as additional antigen presenting cells to activate T cells.

(B) Released surface and intracellular self and tumor antigens form immune complexes with IgG antibodies: plasma cells migrate out of the TLS to the tumor bed along fibroblastic tracks producing CXCL12 and secrete anti-tumor antibodies covering tumor cells. Antibodies trigger tumor cell death either by phagocytosis or by antibody-dependent cell cytotoxicity exerted by macrophages or natural killer (NK) cells. Alternatively, antibodies activate the complement classical pathway leading to tumor cell phagocytosis by macrophages through C3b opsonization, and sustain inflammation by anaphylatoxins generation. Tumor cell death release surface and intracellular self and tumor antigens some forming immune complexes with IgG antibodies.

(C) Activation of immune niches by antigens and IgG-immune complexes: T cells are activated by B cells that have endocytosed antigens via their B cell receptor (BCR) and by myeloid cells after having internalized antigens or IgG-immune complexes.

(D) Reactivation and amplification of the intratumoral immune response in TLS: the anti-tumor T cell response implemented in TLS is re-activated and amplified. T cells are activated either by B cells that have endocytosed antigens through their BCR or by DC that have internalized antigens or immune complexes. Immune complexes also reach the GC, bind to FDC and amplify anti-tumor B cell response. Memory T and B cells, plasma cells and antibodies generated in the tumor can reach systemic circulation and may protect against distant tumor relapse. In most cases, cytotoxic T cells are exhausted and are unable to kill tumor cells. Immune checkpoint inhibition (ICI) therapy overcomes T cell exhaustion in TLS (A), in immune niches (C) and at the contact of tumor cells (bottom left) allowing tumor cell destruction. Anti-PD-1 antibodies may also potentiate Tfh function (A) increasing B cell activation and differentiation. PD-1, programmed death 1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TCR, T cell receptor. Created with biorender.com.

reported in patients with pancreatic cancer vaccinated with irradiated granulocyte-macrophage colony-stimulating factor-secreting allogenic vaccine (GVAX).[118] In the same line, in patients with advanced ovarian cancer treated with a cancer vaccine targeting Wilms' tumor 1 (WT1) antigen, both high anti-WT1 T cell numbers in the blood and high anti-WT1 IgG antibodies in the serum correlated with longer PFS.[119]

Altogether, these studies support the fact that in addition to memory T cells, TLS, and B cells should be considered potential biomarkers to predict patient's responses and to monitor the efficacy of immunotherapies.

B cell involvement in anti-tumor immunity: The intratumoral immune cycle

We propose the following mechanism to support the predictive value of TLS for immunotherapy responses (Figure 2): in mTLS, naive or memory B cells may be triggered by tumor-associated antigens presented by FDCs, activated by signals delivered by Tfh cells and mature toward antibody-producing PCs or more memory B cells.[12] PCs may, however, also be generated in extrafollicular response zones as described in inflamed tissues.[56,120,121] In contrast to GC B cells and plasmablasts that are retained in TLS, PCs travel into the tumor beds as detected in spatial transcriptomic imaging studies. Memory T and B cells as well as PCs may also migrate to peripheral lymphoid organs and the bone marrow where they can be reactivated by a subsequent antigenic challenge delivered by tumor recurrence or metastasis.[12] At the tumor sites, B cells and PCs may impact the functions of the TME in different ways: inside and outside TLS, B cells may present antigen that they have internalized, processed, and associated to MHCII molecules upon recognition via their B cell receptor (BCR) to CD4+ T cells.[120] Antigen presentation via MHCI may also be relevant: B cells in ovarian cancer can present antigenic peptides on MHCI molecules to CD8+ T cells in niches formed with CD8+ T cells.[122] Based on the correlations between antigen-specific B cells in the tumor and IgG bound to tumor cells, it is tempting to speculate that when generated in response to tumor-associated antigens, PCs produce antibodies, some of them binding to tumor cells inducing tumor cell apoptosis upon macrophage activation by tumor bound IgG.[19,55] The resulting immune complexes can be endocytosed by DCs, which present MHCI- and MHCII-associated peptides to CD8+ and CD4+ T cells, respectively, thereby amplifying T cell immunity and diminishing the threshold for stimulation or re-invigoration by immunotherapies. Immune complexes are also taken up by FDC that present the released antigens to B cells in the GC of TLS maintaining and amplifying B cell responses. It may also allow epitope spreading to other tumor-specific and self-antigens, sustaining the autoimmune responses often associated with efficient immunotherapies.[123]

Although attractive, this model is still speculative but opens the way for further focused studies.[28]

The role of IgA antibodies is less clear. They are often associated with poor prognosis,[120] which may be due to the requirement of immunosuppressive TGF-β to induce IgA production.[124] However, an original mechanism has recently been proposed in which polymeric IgA, which do not recognize tumor-specific antigens, are transcytosed in ovarian cancer cells, antagonize the rat sarcoma (RAS) pathway, and sensitize tumor cells to T cell killing.[53] The antibody-mediated mechanisms are therefore diverse, and many of them still need to be uncovered.

ICI may impact the TME at different steps of the intratumoral immunity cycle: inside TLS, anti-PD-1 antibodies may increase Tfh activation to increase the stimulation of B cells and potentially PD-1/PD-L1 blockade, and anti-CTLA-4 antibodies may favor antigen presentation to T cells by DCs. In lymphocyte aggregates forming immune niches, PD-1/PD-L1 blockade allows activation of exhausted T cells by myeloid and B cells. In proximity to tumor cells, ICI reinvigorate T cells, particularly CD8+ T cells, resulting in tumor cell killing. Finally, ICI may also reinvigorate T cells that had been educated in the TME, particularly in TLS, and have nested in peripheral lymph nodes. Interestingly, it was reported that in patients treated with the vascular endothelial growth factor receptor (VEGFR) TKI sunitinib, B cell signature, and binding of IgG antibodies on tumor cells correlated with shorter patient's survival.[125] ccRCC are tumors characterized by a high local production of complement components. C1q produced by infiltrating macrophages binds to IgG-covered tumor cells. The early components of the classical complement cascade (C3, C4) are produced by tumor cells, and upon binding to C1q, they initiate the cascade up to the generation of C3a anaphylatoxins that sustain deleterious chronic inflammation,[125] whereas the tumor cells resist complement killing due to the high levels of inhibitors of late complement components expressed on the cell membrane.[126] In this situation, production of anti-tumor antibodies may result in increased inflammation and vascularization. Classical activation of complement in ccRCC is thus associated with shorter patient survival[126] in a situation where the anti-angiogenic treatment is not able to reinvigorate T cells as ICI.

CONCLUDING REMARKS

TLS are active sites of the generation of anti-tumor immunity when they are located in close vicinity of tumor nests and neighboring tumor-associated antigens. iTLS may be sites of abortive responses with the induction of regulatory lymphocytes that suppress anti-tumor immunity. In mTLS with a GC, full T and B cell immunities are generated or reactivated resulting in effector T cells and antibody production that amplify epitope spreading, further T and B cell activation to tumor-specific and self-antigens. The impact of TLS can therefore be complex, resulting in immune surveillance, lowering the threshold for efficient immunotherapies particularly in tumors with low TMB, and increasing auto-immunity. The most pressing open questions are the relationships between the various immune aggregates and TLS, the relative impacts of TLS and lymph nodes in the generation of anti-tumor immunity, the characterization of tumor-specific or self-antigens recognized by intra-tumoral B cells and antibodies, and the ways to use these data for future immunotherapies. In the future, it will be of paramount importance to dissect these effects in order to better use TLS as markers for predicting efficacious anti-tumor therapies and immune-related adverse events. The aggressiveness of the vast majority of mouse models of cancers with timings too short to allow TLS formation made it difficult to dissect their role in anti-tumor immunity. Today, however, intra-tumoral TLS developed in genetically modified mice[72,127] or upon treatment of tumor-bearing mice with TLS-inducing agents such as LIGHT, CXCL13, LTα, CCL21, TLR4, or CD40 agonists (reviewed in Fridman et al.[64]) or present in tumors grown in inflamed microenvironment such as the peritoneum[128] are being explored. These models will greatly help address specific questions on antigen recognition and subsequent immunity taking place in TLS.

Finally, an extended characterization of the antigens recognized by *in situ*-produced antibodies should also provide new therapeutic antibodies endowed with anti-cancer activities.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Moyron-Quiroz, J.E., Rangel-Moreno, J., Kusser, K., Hartson, L., Sprague, F., Goodrich, S., Woodland, D.L., Lund, F.E., and Randall, T.D. (2004). Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. Nat. Med. *10*, 927–934. https://doi.org/10.1038/nm1091.

2. Aloisi, F., and Pujol-Borrell, R. (2006). Lymphoid neogenesis in chronic inflammatory diseases. Nat. Rev. Immunol. *6*, 205–217. https://doi.org/10.1038/nri1786.

3. Pitzalis, C., Jones, G.W., Bombardieri, M., and Jones, S.A. (2014). Ectopic lymphoid-like structures in infection, cancer and autoimmunity. Nat. Rev. Immunol. *14*, 447–462. https://doi.org/10.1038/nri3700.

4. Thaunat, O., Patey, N., Caligiuri, G., Gautreau, C., Mamani-Matsuda, M., Mekki, Y., Dieu-Nosjean, M.-C., Eberl, G., Ecochard, R., Michel, J.-B., et al. (2010). Chronic rejection triggers the development of an aggressive intragraft immune response through recapitulation of lymphoid organogenesis. J. Immunol. *185*, 717–728. https://doi.org/10.4049/jimmunol.0903589.

5. Moyron-Quiroz, J.E., Rangel-Moreno, J., Hartson, L., Kusser, K., Tighe, M.P., Klonowski, K.D., Lefrançois, L., Cauley, L.S., Harmsen, A.G., Lund, F.E., et al. (2006). Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. Immunity *25*, 643–654. https://doi.org/10.1016/j.immuni.2006.08.022.

6. Manzo, A., Bombardieri, M., Humby, F., and Pitzalis, C. (2010). Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from

inflammation to autoimmunity and tissue damage/remodeling. Immunol. Rev. *233*, 267–285. https://doi.org/10.1111/j.0105-2896.2009.00861.x.

7. Leko, V., and Rosenberg, S.A. (2020). Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. Cancer Cell *38*, 454–472. https://doi.org/10.1016/j.ccell.2020.07.013.

8. Kalaora, S., Nagler, A., Nejman, D., Alon, M., Barbolin, C., Barnea, E., Ketelaars, S.L.C., Cheng, K., Vervier, K., Shental, N., et al. (2021). Identification of bacteria-derived HLA-bound peptides in melanoma. Nature *592*, 138–143. https://doi.org/10.1038/s41586-021-03368-8.

9. Cipponi, A., Mercier, M., Seremet, T., Baurain, J.F., Théate, I., van den Oord, J., Stas, M., Boon, T., Coulie, P.G., and van Baren, N. (2012). Neogenesis of lymphoid structures and antibody responses occur in human melanoma metastases. Cancer Res. *72*, 3997–4007. https://doi.org/10.1158/0008-5472.CAN-12-1377.

10. Dieu-Nosjean, M.-C., Antoine, M., Danel, C., Heudes, D., Wislez, M., Poulot, V., Rabbe, N., Laurans, L., Tartour, E., de Chaisemartin, L., et al. (2008). Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J. Clin. Oncol. *26*, 4410–4417. https://doi.org/10.1200/JCO.2007.15.0284.

11. Chen, D.S., and Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. Immunity *39*, 1–10. https://doi.org/10.1016/j.immuni.2013.07.012.

12. Sautès-Fridman, C., Petitprez, F., Calderaro, J., and Fridman, W.H. (2019). Tertiary lymphoid structures in the era of cancer immunotherapy. Nat. Rev. Cancer *19*, 307–325. https://doi.org/10.1038/s41568-019-0144-6.

13. Fridman, W.H., Meylan, M., Petitprez, F., Sun, C.-M., Italiano, A., and Sautès-Fridman, C. (2022). B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. Nat. Rev. Clin. Oncol. *19*, 441–457. https://doi.org/10.1038/s41571-022-00619-z.

14. Schumacher, T.N., and Thommen, D.S. (2022). Tertiary lymphoid structures in cancer. Science *375*, eabf9419. https://doi.org/10.1126/science.abf9419.

15. Meylan, M., Petitprez, F., Lacroix, L., Di Tommaso, L., Roncalli, M., Bougoüin, A., Laurent, A., Amaddeo, G., Sommacale, D., Regnault, H., et al. (2020). Early hepatic lesions display immature tertiary lymphoid structures and show elevated expression of immune inhibitory and immunosuppressive molecules. Clin. Cancer Res. *26*, 4381–4389. https://doi.org/10.1158/1078-0432.CCR-19-2929.

16. Tietscher, S., Wagner, J., Anzeneder, T., Langwieder, C., Rees, M., Sobottka, B., de Souza, N., and Bodenmiller, B. (2023). A comprehensive single-cell map of T cell exhaustion-associated immune environments in human breast cancer. Nat. Commun. *14*, 98. https://doi.org/10.1038/s41467-022-35238-w.

17. Siliņa, K., Soltermann, A., Attar, F.M., Casanova, R., Uckeley, Z.M., Thut, H., Wandres, M., Isajevs, S., Cheng, P., Curioni-Fontecedro, A., et al. (2018). Germinal centers determine the prognostic relevance of tertiary lymphoid structures and are impaired by corticosteroids in lung squamous cell carcinoma. Cancer Res. *78*, 1308–1320. https://doi.org/10.1158/0008-5472.CAN-17-1987.

18. Vanhersecke, L., Brunet, M., Guégan, J.-P., Rey, C., Bougouin, A., Cousin, S., Le Moulec, S.L., Besse, B., Loriot, Y., Larroquette, M., et al. (2021). Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. Nat. Cancer *2*, 794–802. https://doi.org/10.1038/s43018-021-00232-6.

19. Meylan, M., Petitprez, F., Becht, E., Bougoüin, A., Pupier, G., Calvez, A., Giglioli, I., Verkarre, V., Lacroix, G., Verneau, J., et al. (2022). Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. Immunity *55*, 527–541.e5. https://doi.org/10.1016/j.immuni.2022.02.001.

20. Vanhersecke, L., Bougouin, A., Crombé, A., Brunet, M., Sofeu, C., Parrens, M., Pierron, H., Bonhomme, B., Lembege, N., Rey, C., et al. (2023). Standardized pathology screening of mature tertiary lymphoid structures in cancers. Lab. Invest. *103*, 100063. https://doi.org/10.1016/j.labinv.2023.100063.

21. Barmpoutis, P., Di Capite, M., Kayhanian, H., Waddingham, W., Alexander, D.C., Jansen, M., and Kwong, F.N.K. (2021). Tertiary lymphoid structures (TLS) identification and density assessment on H&E-stained digital slides of lung cancer. PLoS One *16*, e0256907. https://doi.org/10.1371/journal.pone.0256907.

22. Hu, J., Coleman, K., Zhang, D., Lee, E.B., Kadara, H., Wang, L., and Li, M. (2023). Deciphering tumor ecosystems at super resolution from spatial transcriptomics with TESLA. Cell Syst. *14*, 404–417.e4. https://doi.org/10.1016/j.cels.2023.03.008.

23. Petitprez, F., de Reyniès, A., Keung, E.Z., Chen, T.W.-W., Sun, C.-M., Calderaro, J., Jeng, Y.-M., Hsiao, L.-P., Lacroix, L., Bougoüin, A., et al. (2020). B cells are associated with survival and immunotherapy response in sarcoma. Nature *577*, 556–560. https://doi.org/10.1038/s41586-019-1906-8.

24. Bindea, G., Mlecnik, B., Tosolini, M., Kirilovsky, A., Waldner, M., Obenauf, A.C., Angell, H., Fredriksen, T., Lafontaine, L., Berger, A., et al. (2013). Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity *39*, 782–795. https://doi.org/10.1016/j.immuni.2013.10.003.

25. Coppola, D., Nebozhyn, M., Khalil, F., Dai, H., Yeatman, T., Loboda, A., and Mulé, J.J. (2011). Unique ectopic lymph node-like structures present in human primary colorectal carcinoma are identified by immune gene array profiling. Am. J. Pathol. *179*, 37–45. https://doi.org/10.1016/j.ajpath.2011.03.007.

26. Gu-Trantien, C., Loi, S., Garaud, S., Equeter, C., Libin, M., de Wind, A., Ravoet, M., Le Buanec, H., Sibille, C., Manfouo-Foutsop, G., et al. (2013). $CD4^+$ follicular helper T cell infiltration predicts breast cancer survival. J. Clin. Invest. *123*, 2873–2892. https://doi.org/10.1172/JCI67428.

27. Hennequin, A., Derangère, V., Boidot, R., Apetoh, L., Vincent, J., Orry, D., Fraisse, J., Causeret, S., Martin, F., Arnould, L., et al. (2016). Tumor infiltration by Tbet+ effector T cells and CD20+ B cells is associated with survival in gastric cancer patients. Oncoimmunology *5*, e1054598. https://doi.org/10.1080/2162402X.2015.1054598.

28. Kroeger, D.R., Milne, K., and Nelson, B.H. (2016). Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. Clin. Cancer Res. *22*, 3005–3015. https://doi.org/10.1158/1078-0432.CCR-15-2762.

29. Becht, E., Giraldo, N.A., Lacroix, L., Buttard, B., Elarouci, N., Petitprez, F., Selves, J., Laurent-Puig, P., Sautès-Fridman, C., Fridman, W.H., et al. (2016). Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol. *17*, 218. https://doi.org/10.1186/s13059-016-1070-5.

30. Zhang, H., AbdulJabbar, K., Moore, D.A., Akarca, A., Enfield, K.S.S., Jamal-Hanjani, M., Raza, S.E.A., Veeriah, S., Salgado, R., McGranahan, N., et al. (2023). Spatial positioning of immune hotspots reflects the interplay between B and T cells in lung squamous cell carcinoma. Cancer Res. *83*, 1410–1425. https://doi.org/10.1158/0008-5472.CAN-22-2589.

31. Mi, H., Sivagnanam, S., Betts, C.B., Liudahl, S.M., Jaffee, E.M., Coussens, L.M., and Popel, A.S. (2022). Quantitative spatial profiling of immune populations in pancreatic ductal adenocarcinoma reveals tumor microenvironment heterogeneity and prognostic biomarkers. Cancer Res. *82*, 4359–4372. https://doi.org/10.1158/0008-5472.CAN-22-1190.

32. Hao, D., Han, G., Sinjab, A., Gomez-Bolanos, L.I., Lazcano, R., Serrano, A., Hernandez, S.D., Dai, E., Cao, X., Hu, J., et al. (2022). The single-cell immunogenomic landscape of B and plasma cells in early-stage lung adenocarcinoma. Cancer Discov. *12*, 2626–2645. https://doi.org/10.1158/2159-8290.CD-21-1658.

33. Leader, A.M., Grout, J.A., Maier, B.B., Nabet, B.Y., Park, M.D., Tabachnikova, A., Chang, C., Walker, L., Lansky, A., Le Berichel, J., et al. (2021). Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. Cancer Cell *39*, 1594–1609.e12. https://doi.org/10.1016/j.ccell.2021.10.009.

34. Gaglia, G., Burger, M.L., Ritch, C.C., Rammos, D., Dai, Y., Crossland, G.E., Tavana, S.Z., Warchol, S., Jaeger, A.M., Naranjo, S., et al. (2023). Lymphocyte networks are dynamic cellular communities in the immunoregulatory landscape of lung adenocarcinoma. Cancer Cell *41*, 871–886.e10. https://doi.org/10.1016/j.ccell.2023.03.015.

35. Magen, A., Hamon, P., Fiaschi, N., Soong, B.Y., Park, M.D., Mattiuz, R., Humblin, E., Troncoso, L., D'Souza, D., Dawson, T., et al. (2023). Intratumoral dendritic cell–CD4+ T helper cell niches enable CD8+ T cell differentiation following PD-1 blockade in hepatocellular carcinoma. Nat. Med. *29*, 1389–1399. https://doi.org/10.1038/s41591-023-02345-0.

36. Hoch, T., Schulz, D., Eling, N., Gómez, J.M., Levesque, M.P., and Bodenmiller, B. (2022). Multiplexed imaging mass cytometry of the chemokine milieus in melanoma characterizes features of the response to immunotherapy. Sci. Immunol. *7*, eabk1692. https://doi.org/10.1126/sciimmunol.abk1692.

37. Cohen, M., Giladi, A., Barboy, O., Hamon, P., Li, B., Zada, M., Gurevich-Shapiro, A., Beccaria, C.G., David, E., Maier, B.B., et al. (2022). The interaction of CD4+ helper T cells with dendritic cells shapes the tumor microenvironment and immune checkpoint blockade response. Nat. Cancer *3*, 303–317. https://doi.org/10.1038/s43018-022-00338-5.

38. Pelka, K., Hofree, M., Chen, J.H., Sarkizova, S., Pirl, J.D., Jorgji, V., Bejnood, A., Dionne, D., Ge, W.H., Xu, K.H., et al. (2021). Spatially organized multicellular immune hubs in human colorectal cancer. Cell *184*, 4734–4752.e20. https://doi.org/10.1016/j.cell.2021.08.003.

39. Chen, J.H., Nieman, L.T., Spurrell, M., Jorgji, V., Richieri, P., Xu, K.H., Madhu, R., Parikh, M., Zamora, I., Mehta, A., et al. (2023). Spatial analysis of human lung cancer reveals organized immune hubs enriched for stem-like CD8 T cells and associated with immunotherapy response. https://doi.org/10.1101/2023.04.04.535379.

40. Jansen, C.S., Prokhnevska, N., Master, V.A., Sanda, M.G., Carlisle, J.W., Bilen, M.A., Cardenas, M., Wilkinson, S., Lake, R., Sowalsky, A.G., et al. (2019). An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. Nature *576*, 465–470. https://doi.org/10.1038/s41586-019-1836-5.

41. Siddiqui, I., Schaeuble, K., Chennupati, V., Fuertes Marraco, S.A., Calderon-Copete, S., Pais Ferreira, D., Carmona, S.J., Scarpellino, L., Gfeller, D., Pradervand, S., et al. (2019). Intratumoral Tcf1+PD-1+CD8+ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. Immunity *50*, 195–211.e10. https://doi.org/10.1016/j.immuni.2018.12.021.

42. Hua, Y., Vella, G., Rambow, F., Allen, E., Antoranz Martinez, A., Duhamel, M., Takeda, A., Jalkanen, S., Junius, S., Smeets, A., et al. (2022). Cancer immunotherapies transition endothelial cells into HEVs that generate TCF1+ T lymphocyte niches through a feed-forward loop. Cancer Cell *40*, 1600–1618.e10. https://doi.org/10.1016/j.ccell.2022.11.002.

43. Stoltzfus, C.R., Sivakumar, R., Kunz, L., Olin Pope, B.E., Menietti, E., Speziale, D., Adelfio, R., Bacac, M., Colombetti, S., Perro, M., et al. (2021). Multi-parameter quantitative imaging of tumor microenvironments reveals perivascular immune niches associated with anti-tumor immunity. Front. Immunol. *12*, 726492. https://doi.org/10.3389/fimmu.2021.726492.

44. Chu, Y., Dai, E., Li, Y., Han, G., Pei, G., Ingram, D.R., Thakkar, K., Qin, J.J., Dang, M., Le, X., et al. (2023). Pan-cancer T cell atlas links a cellular stress response state to immunotherapy resistance. Nat. Med. *29*, 1550–1562. https://doi.org/10.1038/s41591-023-02371-y.

45. Goc, J., Germain, C., Vo-Bourgais, T.K.D., Lupo, A., Klein, C., Knockaert, S., de Chaisemartin, L., Ouakrim, H., Becht, E., Alifano, M., et al. (2014). Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res. *74*, 705–715. https://doi.org/10.1158/0008-5472.CAN-13-1342.

46. Prokhnevska, N., Cardenas, M.A., Valanparambil, R.M., Sobierajska, E., Barwick, B.G., Jansen, C., Reyes Moon, A., Gregorova, P., delBalzo, L., Greenwald, R., et al. (2023). CD8+ T cell activation in cancer comprises an initial activation phase in lymph nodes followed by effector differentiation within the tumor. Immunity *56*, 107–124.e5. https://doi.org/10.1016/j.immuni.2022.12.002.

47. Asrir, A., Tardiveau, C., Coudert, J., Laffont, R., Blanchard, L., Bellard, E., Veerman, K., Bettini, S., Lafouresse, F., Vina, E., et al. (2022). Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy. Cancer Cell *40*, 318–334.e9. https://doi.org/10.1016/j.ccell.2022.01.002.

48. Calderaro, J., Petitprez, F., Becht, E., Laurent, A., Hirsch, T.Z., Rousseau, B., Luciani, A., Amaddeo, G., Derman, J., Charpy, C., et al. (2018). Intratumoral tertiary lymphoid structures are associated with a low risk of early recurrence of hepatocellular carcinoma. J. Hepatol. *70*, 58–65. https://doi.org/10.1016/j.jhep.2018.09.003.

49. Finkin, S., Yuan, D., Stein, I., Taniguchi, K., Weber, A., Unger, K., Browning, J.L., Goossens, N., Nakagawa, S., Gunasekaran, G., et al. (2015). Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. Nat. Immunol. *16*, 1235–1244. https://doi.org/10.1038/ni.3290.

50. van Dijk, N., Gil-Jimenez, A., Silina, K., van Montfoort, M.L., Einerhand, S., Jonkman, L., Voskuilen, C.S., Peters, D., Sanders, J., Lubeck, Y., et al. (2021). The tumor immune landscape and architecture of tertiary lymphoid structures in urothelial cancer. Front. Immunol. *12*, 793964. https://doi.org/10.3389/fimmu.2021.793964.

51. Montfort, A., Pearce, O., Maniati, E., Vincent, B.G., Bixby, L., Böhm, S., Dowe, T., Wilkes, E.H., Chakravarty, P., Thompson, R., et al. (2017). A strong B-cell response is part of the immune landscape in human high-grade serous ovarian metastases. Clin. Cancer Res. *23*, 250–262. https://doi.org/10.1158/1078-0432.CCR-16-0081.

52. Wouters, M.C.A., and Nelson, B.H. (2018). Prognostic significance of tumor-infiltrating B cells and plasma cells in human cancer. Clin. Cancer Res. *24*, 6125–6135. https://doi.org/10.1158/1078-0432.CCR-18-1481.

53. Biswas, S., Mandal, G., Payne, K.K., Anadon, C.M., Gatenbee, C.D., Chaurio, R.A., Costich, T.L., Moran, C., Harro, C.M., Rigolizzo, K.E., et al. (2021). IgA transcytosis and antigen recognition govern ovarian cancer immunity. Nature *591*, 464–470. https://doi.org/10.1038/s41586-020-03144-0.

54. Italiano, A., Bessede, A., Pulido, M., Bompas, E., Piperno-Neumann, S., Chevreau, C., Penel, N., Bertucci, F., Toulmonde, M., Bellera, C., et al. (2022). Pembrolizumab in soft-tissue sarcomas with tertiary lymphoid structures: a phase 2 PEMBROSARC trial cohort. Nat. Med. *28*, 1199–1206. https://doi.org/10.1038/s41591-022-01821-3.

55. Mazor, R.D., Nathan, N., Gilboa, A., Stoler-Barak, L., Moss, L., Solomonov, I., Hanuna, A., Divinsky, Y., Shmueli, M.D., Hezroni, H., et al. (2022). Tumor-reactive antibodies evolve from non-binding and autoreactive precursors. Cell *185*, 1208–1222.e21. https://doi.org/10.1016/j.cell.2022.02.012.

56. Laumont, C.M., Banville, A.C., Gilardi, M., Hollern, D.P., and Nelson, B.H. (2022). Tumour-infiltrating B cells: immunological mechanisms, clinical impact and therapeutic opportunities. Nat. Rev. Cancer *22*, 414–430. https://doi.org/10.1038/s41568-022-00466-1.

57. Fridman, W.H., Sibéril, S., Pupier, G., Soussan, S., and Sautès-Fridman, C. (2023). Activation of B cells in tertiary Lymphoid Structures in cancer: anti-tumor or anti-self? Semin. Immunol. *65*, 101703. https://doi.org/10.1016/j.smim.2022.101703.

58. Chaurio, R.A., Anadon, C.M., Lee Costich, T., Payne, K.K., Biswas, S., Harro, C.M., Moran, C., Ortiz, A.C., Cortina, C., Rigolizzo, K.E., et al. (2022). TGF-β-mediated silencing of genomic organizer SATB1 promotes Tfh cell differentiation and formation of intra-tumoral tertiary lymphoid structures. Immunity *55*, 115–128.e9. https://doi.org/10.1016/j.immuni.2021.12.007.

59. Germain, C., Gnjatic, S., Tamzalit, F., Knockaert, S., Remark, R., Goc, J., Lepelley, A., Becht, E., Katsahian, S., Bizouard, G., et al. (2014). Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am. J. Respir. Crit. Care Med. *189*, 832–844. https://doi.org/10.1164/rccm.201309-1611OC.

60. Wieland, A., Patel, M.R., Cardenas, M.A., Eberhardt, C.S., Hudson, W.H., Obeng, R.C., Griffith, C.C., Wang, X., Chen, Z.G., Kissick, H.T., et al. (2021). Defining HPV-specific B cell responses in patients with head and neck cancer. Nature *597*, 274–278. https://doi.org/10.1038/s41586-020-2931-3.

61. Ng, K.W., Boumelha, J., Enfield, K.S.S., Almagro, J., Cha, H., Pich, O., Karasaki, T., Moore, D.A., Salgado, R., Sivakumar, M., et al. (2023). Antibodies against endogenous retroviruses promote lung cancer immunotherapy. Nature *616*, 563–573. https://doi.org/10.1038/s41586-023-05771-9.

62. Pavoni, E., Monteriù, G., Santapaola, D., Petronzelli, F., Anastasi, A.M., Pelliccia, A., D'Alessio, V., De Santis, R.D., and Minenkova, O. (2007). Tumor-infiltrating B lymphocytes as an efficient source of highly specific immunoglobulins recognizing tumor cells. BMC Biotechnol. *7*, 70. https://doi.org/10.1186/1472-6750-7-70.

63. Garaud, S., Zayakin, P., Buisseret, L., Rulle, U., Silina, K., de Wind, A., Van den Eyden, G., Larsimont, D., Willard-Gallo, K., and Linē, A. (2018). Antigen specificity and clinical significance of IgG and IgA autoantibodies produced in situ by tumor-infiltrating B cells in breast cancer. Front. Immunol. *9*, 2660. https://doi.org/10.3389/fimmu.2018.02660.

64. Fridman, W.H., Zitvogel, L., Sautès-Fridman, C., and Kroemer, G. (2017). The immune contexture in cancer prognosis and treatment. Nat. Rev. Clin. Oncol. *14*, 717–734. https://doi.org/10.1038/nrclinonc.2017.101.

65. Remark, R., Becker, C., Gomez, J.E., Damotte, D., Dieu-Nosjean, M.-C., Sautès-Fridman, C., Fridman, W.-H., Powell, C.A., Altorki, N.K., Merad, M., et al. (2015). The non-small cell lung cancer immune contexture. A major determinant of tumor characteristics and patient outcome. Am. J. Respir. Crit. Care Med. *191*, 377–390. https://doi.org/10.1164/rccm.201409-1671PP.

66. Fridman, W.H., Pagès, F., Sautès-Fridman, C., and Galon, J. (2012). The immune contexture in human tumours: impact on clinical outcome. Nat. Rev. Cancer *12*, 298–306. https://doi.org/10.1038/nrc3245.

67. Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., Tosolini, M., Camus, M., Berger, A., Wind, P., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science *313*, 1960–1964. https://doi.org/10.1126/science.1129139.

68. Bruni, D., Angell, H.K., and Galon, J. (2020). The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nat. Rev. Cancer *20*, 662–680. https://doi.org/10.1038/s41568-020-0285-7.

69. Oliveira, G., and Wu, C.J. (2023). Dynamics and specificities of T cells in cancer immunotherapy. Nat. Rev. Cancer *23*, 295–316. https://doi.org/10.1038/s41568-023-00560-y.

70. Devi-Marulkar, P., Fastenackels, S., Karapentiantz, P., Goc, J., Germain, C., Kaplon, H., Knockaert, S., Olive, D., Panouillot, M., Validire, P., et al. (2022). Regulatory T cells infiltrate the tumor-induced tertiary lymphoïd structures and are associated with poor clinical outcome in NSCLC. Commun. Biol. *5*, 1416. https://doi.org/10.1038/s42003-022-04356-y.

71. Schweiger, T., Berghoff, A.S., Glogner, C., Glueck, O., Rajky, O., Traxler, D., Birner, P., Preusser, M., Klepetko, W., and Hoetzenecker, K. (2016). Tumor-infiltrating lymphocyte subsets and tertiary lymphoid structures in pulmonary metastases from colorectal cancer. Clin. Exp. Metastasis *33*, 727–739. https://doi.org/10.1007/s10585-016-9813-y.

72. Joshi, N.S., Akama-Garren, E.H., Lu, Y., Lee, D.-Y., Chang, G.P., Li, A., DuPage, M., Tammela, T., Kerper, N.R., Farago, A.F., et al. (2015). Regulatory T cells in tumor-associated tertiary lymphoid structures suppress anti-tumor T cell responses. Immunity *43*, 579–590. https://doi.org/10.1016/j.immuni.2015.08.006.

73. Overacre-Delgoffe, A.E., Bumgarner, H.J., Cillo, A.R., Burr, A.H.P., Tometich, J.T., Bhattacharjee, A., Bruno, T.C., Vignali, D.A.A., and Hand, T.W. (2021). Microbiota-specific T follicular helper cells drive tertiary lymphoid structures and anti-tumor immunity against colorectal cancer. Immunity *54*, 2812–2824.e4. https://doi.org/10.1016/j.immuni.2021.11.003.

74. Cillo, A.R., Kürten, C.H.L., Tabib, T., Qi, Z., Onkar, S., Wang, T., Liu, A., Duvvuri, U., Kim, S., Soose, R.J., et al. (2020). Immune landscape of viral- and carcinogen-driven head and neck cancer. Immunity *52*, 183–199.e9. https://doi.org/10.1016/j.immuni.2019.11.014.

75. Shalapour, S., Lin, X.-J., Bastian, I.N., Brain, J., Burt, A.D., Aksenov, A.A., Vrbanac, A.F., Li, W., Perkins, A., Matsutani, T., et al. (2017). Inflammation-induced IgA + cells dismantle anti-liver cancer immunity. Nature *551*, 340–345. https://doi.org/10.1038/nature24302.

76. de Visser, K.E., Korets, L.V., and Coussens, L.M. (2005). De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell *7*, 411–423. https://doi.org/10.1016/j.ccr.2005.04.014.

77. Brodt, P., and Gordon, J. (1978). Anti-tumor immunity in B lymphocyte-deprived mice. I. Immunity to a chemically induced tumor. J. Immunol. *121*, 359–362.

78. Barbera-Guillem, E., Nelson, M.B., Barr, B., Nyhus, J.K., May, K.F., Feng, L., and Sampsel, J.W. (2000). B lymphocyte pathology in human colorectal cancer. Experimental and clinical therapeutic effects of partial B cell depletion. Cancer Immunol. Immunother. *48*, 541–549. https://doi.org/10.1007/pl00006672.

79. Qin, Z., Richter, G., Schüler, T., Ibe, S., Cao, X., and Blankenstein, T. (1998). B cells inhibit induction of T cell-dependent tumor immunity. Nat. Med. *4*, 627–630. https://doi.org/10.1038/nm0598-627.

80. Shah, S., Divekar, A.A., Hilchey, S.P., Cho, H.-M., Newman, C.L., Shin, S.-U., Nechustan, H., Challita-Eid, P.M., Segal, B.M., Yi, K.H., et al. (2005). Increased rejection of primary tumors in mice lacking B cells: inhibition of anti-tumor CTL and TH1 cytokine responses by B cells. Int. J. Cancer *117*, 574–586. https://doi.org/10.1002/ijc.21177.

81. Fridman, W.H., Petitprez, F., Meylan, M., Chen, T.W.-W., Sun, C.-M., Roumenina, L.T., and Sautès-Fridman, C. (2021). B cells and cancer: to B or not to B? J. Exp. Med. *218*, e20200851. https://doi.org/10.1084/jem.20200851.

82. Michaud, D., Steward, C.R., Mirlekar, B., and Pylayeva-Gupta, Y. (2021). Regulatory B cells in cancer. Immunol. Rev. *299*, 74–92. https://doi.org/10.1111/imr.12939.

83. Shao, Y., Lo, C.M., Ling, C.C., Liu, X.B., Ng, K.T.-P., Chu, A.C.Y., Ma, Y.Y., Li, C.X., Fan, S.T., and Man, K. (2014). Regulatory B cells accelerate hepatocellular carcinoma progression via CD40/CD154 signaling pathway. Cancer Lett. *355*, 264–272. https://doi.org/10.1016/j.canlet.2014.09.026.

84. Zhou, X., Su, Y.-X., Lao, X.-M., Liang, Y.-J., and Liao, G.-Q. (2016). CD19+IL-10+ regulatory B cells affect survival of tongue squamous cell carcinoma patients and induce resting CD4+ T cells to CD4+Foxp3+ regulatory T cells. Oral Oncol. *53*, 27–35. https://doi.org/10.1016/j.oraloncology.2015.11.003.

85. Murakami, Y., Saito, H., Shimizu, S., Kono, Y., Shishido, Y., Miyatani, K., Matsunaga, T., Fukumoto, Y., Ashida, K., Sakabe, T., et al. (2019). Increased regulatory B cells are involved in immune evasion in patients with gastric cancer. Sci. Rep. *9*, 13083. https://doi.org/10.1038/s41598-019-49581-4.

86. Ishigami, E., Sakakibara, M., Sakakibara, J., Masuda, T., Fujimoto, H., Hayama, S., Nagashima, T., Sangai, T., Nakagawa, A., Nakatani, Y., et al. (2019). Coexistence of regulatory B cells and regulatory T cells in tumor-infiltrating lymphocyte aggregates is a prognostic factor in patients with breast cancer. Breast Cancer *26*, 180–189. https://doi.org/10.1007/s12282-018-0910-4.

87. Mirlekar, B., Wang, Y., Li, S., Zhou, M., Entwistle, S., De Buysscher, T., Morrison, A., Herrera, G., Harris, C., Vincent, B.G., et al. (2022). Balance between immunoregulatory B cells and plasma cells drives pancreatic tumor immunity. Cell Rep. Med. *3*, 100744. https://doi.org/10.1016/j.xcrm.2022.100744.

88. Li, S., Mirlekar, B., Johnson, B.M., Brickey, W.J., Wrobel, J.A., Yang, N., Song, D., Entwistle, S., Tan, X., Deng, M., et al. (2022). STING-induced regulatory B cells compromise NK function in cancer immunity. Nature *610*, 373–380. https://doi.org/10.1038/s41586-022-05254-3.

89. de Chaisemartin, L., Goc, J., Damotte, D., Validire, P., Magdeleinat, P., Alifano, M., Cremer, I., Fridman, W.-H., Sautès-Fridman, C., and Dieu-Nosjean, M.-C. (2011). Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res. *71*, 6391–6399. https://doi.org/10.1158/0008-5472.CAN-11-0952.

90. Ruffin, A.T., Cillo, A.R., Tabib, T., Liu, A., Onkar, S., Kunning, S.R., Lampenfeld, C., Atiya, H.I., Abecassis, I., Kürten, C.H.L., et al. (2021). B cell signatures and tertiary lymphoid structures contribute to outcome in head and neck squamous cell carcinoma. Nat. Commun. *12*, 3349. https://doi.org/10.1038/s41467-021-23355-x.

91. Weiner, A.B., Vidotto, T., Liu, Y., Mendes, A.A., Salles, D.C., Faisal, F.A., Murali, S., McFarlane, M., Imada, E.L., Zhao, X., et al. (2021). Plasma cells are enriched in localized prostate cancer in Black men and are

associated with improved outcomes. Nat. Commun. *12*, 935. https://doi.org/10.1038/s41467-021-21245-w.

92. Sharma, P., and Allison, J.P. (2015). The future of immune checkpoint therapy. Science *348*, 56–61. https://doi.org/10.1126/science.aaa8172.

93. Hirsch, L., Zitvogel, L., Eggermont, A., and Marabelle, A. (2019). PD-Loma: a cancer entity with a shared sensitivity to the PD-1/PD-L1 pathway blockade. Br. J. Cancer *120*, 3–5. https://doi.org/10.1038/s41416-018-0294-4.

94. Tumeh, P.C., Harview, C.L., Yearley, J.H., Shintaku, I.P., Taylor, E.J.M., Robert, L., Chmielowski, B., Spasic, M., Henry, G., Ciobanu, V., et al. (2014). PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature *515*, 568–571. https://doi.org/10.1038/nature13954.

95. de Vries, N.L., van de Haar, J., Veninga, V., Chalabi, M., Ijsselsteijn, M.E., van der Ploeg, M., van den Bulk, J., Ruano, D., van den Berg, J.G., Haanen, J.B., et al. (2023). γδ T cells are effectors of immunotherapy in cancers with HLA class I defects. Nature *613*, 743–750. https://doi.org/10.1038/s41586-022-05593-1.

96. Le, D.T., Durham, J.N., Smith, K.N., Wang, H., Bartlett, B.R., Aulakh, L.K., Lu, S., Kemberling, H., Wilt, C., Luber, B.S., et al. (2017). Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science *357*, 409–413. https://doi.org/10.1126/science.aan6733.

97. Le, D.T., Uram, J.N., Wang, H., Bartlett, B.R., Kemberling, H., Eyring, A.D., Skora, A.D., Luber, B.S., Azad, N.S., Laheru, D., et al. (2015). PD-1 blockade in tumors with mismatch-repair deficiency. N. Engl. J. Med. *372*, 2509–2520. https://doi.org/10.1056/NEJMoa1500596.

98. Chalabi, M., Fanchi, L.F., Dijkstra, K.K., Van den Berg, J.G., Aalbers, A.G., Sikorska, K., Lopez-Yurda, M., Grootscholten, C., Beets, G.L., Snaebjornsson, P., et al. (2020). Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat. Med. *26*, 566–576. https://doi.org/10.1038/s41591-020-0805-8.

99. Cercek, A., Lumish, M., Sinopoli, J., Weiss, J., Shia, J., Lamendola-Essel, M., El Dika, I.H., Segal, N., Shcherba, M., Sugarman, R., et al. (2022). PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. N. Engl. J. Med. *386*, 2363–2376. https://doi.org/10.1056/NEJMoa2201445.

100. Gao, J., Navai, N., Alhalabi, O., Siefker-Radtke, A., Campbell, M.T., Tidwell, R.S., Guo, C.C., Kamat, A.M., Matin, S.F., Araujo, J.C., et al. (2020). Neoadjuvant PD-L1 plus CTLA-4 blockade in patients with cisplatin-ineligible operable high-risk urothelial carcinoma. Nat. Med. *26*, 1845–1851. https://doi.org/10.1038/s41591-020-1086-y.

101. van Dijk, N., Gil-Jimenez, A., Silina, K., Hendricksen, K., Smit, L.A., de Feijter, J.M., van Montfoort, M.L., van Rooijen, C., Peters, D., Broeks, A., et al. (2020). Preoperative ipilimumab plus nivolumab in locoregionally advanced urothelial cancer: the NABUCCO trial. Nat. Med. *26*, 1839–1844. https://doi.org/10.1038/s41591-020-1085-z.

102. Cottrell, T.R., Thompson, E.D., Forde, P.M., Stein, J.E., Duffield, A.S., Anagnostou, V., Rekhtman, N., Anders, R.A., Cuda, J.D., Illei, P.B., et al. (2018). Pathologic features of response to neoadjuvant anti-PD-1 in resected non-small-cell lung carcinoma: a proposal for quantitative immune-related pathologic response criteria (irPRC). Ann. Oncol. *29*, 1853–1860. https://doi.org/10.1093/annonc/mdy218.

103. Cascone, T., Leung, C.H., Weissferdt, A., Pataer, A., Carter, B.W., Godoy, M.C.B., Feldman, H., William, W.N., Xi, Y., Basu, S., et al. (2023). Neoadjuvant chemotherapy plus nivolumab with or without ipilimumab in operable non-small cell lung cancer: the phase 2 platform NEOSTAR trial. Nat. Med. *29*, 593–604. https://doi.org/10.1038/s41591-022-02189-0.

104. Powles, T., Kockx, M., Rodriguez-Vida, A., Duran, I., Crabb, S.J., Van Der Heijden, M.S., Szabados, B., Pous, A.F., Gravis, G., Herranz, U.A., et al. (2019). Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable urothelial carcinoma in the ABACUS trial. Nat. Med. *25*, 1706–1714. https://doi.org/10.1038/s41591-019-0628-7.

105. Vos, J.L., Elbers, J.B.W., Krijgsman, O., Traets, J.J.H., Qiao, X., van der Leun, A.M., Lubeck, Y., Seignette, I.M., Smit, L.A., Willems, S.M., et al. (2021). Neoadjuvant immunotherapy with nivolumab and ipilimumab induces major pathological responses in patients with head and neck squamous cell carcinoma. Nat. Commun. *12*, 7348. https://doi.org/10.1038/s41467-021-26472-9.

106. Cabrita, R., Lauss, M., Sanna, A., Donia, M., Skaarup Larsen, M.S., Mitra, S., Johansson, I., Phung, B., Harbst, K., Vallon-Christersson, J., et al. (2020). Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature *577*, 561–565. https://doi.org/10.1038/s41586-019-1914-8.

107. Dunn, G.P., Old, L.J., and Schreiber, R.D. (2004). The three Es of cancer immunoediting. Annu. Rev. Immunol. *22*, 329–360. https://doi.org/10.1146/annurev.immunol.22.012703.104803.

108. Schmidt, J., Smith, A.R., Magnin, M., Racle, J., Devlin, J.R., Bobisse, S., Cesbron, J., Bonnet, V., Carmona, S.J., Huber, F., et al. (2021). Prediction of neo-epitope immunogenicity reveals TCR recognition determinants and provides insight into immunoediting. Cell Rep. Med. *2*, 100194. https://doi.org/10.1016/j.xcrm.2021.100194.

109. Rosenthal, R., Cadieux, E.L., Salgado, R., Bakir, M.A., Moore, D.A., Hiley, C.T., Lund, T., Tanić, M., Reading, J.L., Joshi, K., et al. (2019). Neoantigen-directed immune escape in lung cancer evolution. Nature *567*, 479–485. https://doi.org/10.1038/s41586-019-1032-7.

110. Litchfield, K., Reading, J.L., Puttick, C., Thakkar, K., Abbosh, C., Bentham, R., Watkins, T.B.K., Rosenthal, R., Biswas, D., Rowan, A., et al. (2021). Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. Cell *184*, 596–614.e14. https://doi.org/10.1016/j.cell.2021.01.002.

111. Helmink, B.A., Reddy, S.M., Gao, J., Zhang, S., Basar, R., Thakur, R., Yizhak, K., Sade-Feldman, M., Blando, J., Han, G., et al. (2020). B cells and tertiary lymphoid structures promote immunotherapy response. Nature *577*, 549–555. https://doi.org/10.1038/s41586-019-1922-8.

112. Vano, Y.-A., Elaidi, R., Bennamoun, M., Chevreau, C., Borchiellini, D., Pannier, D., Maillet, D., Gross-Goupil, M., Tournigand, C., Laguerre, B., et al. (2022). Nivolumab, nivolumab-ipilimumab, and VEGFR-tyrosine kinase inhibitors as first-line treatment for metastatic clear-cell renal cell carcinoma (BIONIKK): a biomarker-driven, open-label, non-comparative, randomised, phase 2 trial. Lancet Oncol. *23*, 612–624. https://doi.org/10.1016/S1470-2045(22)00128-0.

113. White, K., Connor, K., Meylan, M., Bougoüin, A., Salvucci, M., Bielle, F., O'Farrell, A.C., Sweeney, K., Weng, L., Bergers, G., et al. (2023). Identification, validation and biological characterisation of novel glioblastoma tumour microenvironment subtypes: implications for precision immunotherapy. Ann. Oncol. *34*, 300–314. https://doi.org/10.1016/j.annonc.2022.11.008.

114. Bruno, T.C. (2020). New predictors for immunotherapy responses sharpen our view of the tumour microenvironment. Nature *577*, 474–476. https://doi.org/10.1038/d41586-019-03943-0.

115. Patil, N.S., Nabet, B.Y., Müller, S., Koeppen, H., Zou, W., Giltnane, J., Au-Yeung, A., Srivats, S., Cheng, J.H., Takahashi, C., et al. (2022). Intratumoral plasma cells predict outcomes to PD-L1 blockade in non-small cell lung cancer. Cancer Cell *40*, 289–300.e4. https://doi.org/10.1016/j.ccell.2022.02.002.

116. Toulmonde, M., Penel, N., Adam, J., Chevreau, C., Blay, J.-Y., Le Cesne, A., Bompas, E., Piperno-Neumann, S., Cousin, S., Grellety, T., et al. (2018). Use of PD-1 targeting, macrophage infiltration, and IDO pathway activation in sarcomas: A Phase 2 clinical trial. JAMA Oncol. *4*, 93–97. https://doi.org/10.1001/jamaoncol.2017.1617.

117. Maldonado, L., Teague, J.E., Morrow, M.P., Jotova, I., Wu, T.C., Wang, C., Desmarais, C., Boyer, J.D., Tycko, B., Robins, H.S., et al. (2014). Intramuscular therapeutic vaccination targeting HPV16 induces T cell responses that localize in mucosal lesions. Sci. Transl. Med. *6*, 221ra13. https://doi.org/10.1126/scitranslmed.3007323.

118. Lutz, E.R., Wu, A.A., Bigelow, E., Sharma, R., Mo, G., Soares, K., Solt, S., Dorman, A., Wamwea, A., Yager, A., et al. (2014). Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol. Res. *2*, 616–631. https://doi.org/10.1158/2326-6066.CIR-14-0027.

119. Nishida, S., Morimoto, S., Oji, Y., Morita, S., Shirakata, T., Enomoto, T., Tsuboi, A., Ueda, Y., Yoshino, K., Shouq, A., et al. (2022). Cellular and humoral immune responses induced by an HLA class I-restricted peptide

cancer vaccine targeting WT1 are associated with favorable clinical outcomes in advanced ovarian cancer. J. Immunother. *45*, 56–66. https://doi.org/10.1097/CJI.0000000000000405.

120. Laumont, C.M., and Nelson, B.H. (2023). B cells in the tumor microenvironment: multi-faceted organizers, regulators, and effectors of anti-tumor immunity. Cancer Cell *41*, 466–489. https://doi.org/10.1016/j.ccell.2023.02.017.

121. Koiwa, Y., Kikuchi, J.I., Takagi, T., Honda, H., and Hoshi, N. (1989). Human left ventricular wall vibration responded to precordial minute vibration. Tohoku J Exp Med *159*, 79–80. https://doi.org/10.1620/tjem.159.79.

122. Hladíková, K., Koucký, V., Bouček, J., Laco, J., Grega, M., Hodek, M., Zábrodský, M., Vošmik, M., Rozkošová, K., Vošmiková, H., et al. (2019). Tumor-infiltrating B cells affect the progression of oropharyngeal squamous cell carcinoma via cell-to-cell interactions with CD8+ T cells. J. Immunother. Cancer *7*, 261. https://doi.org/10.1186/s40425-019-0726-6.

123. Zhou, X., Yao, Z., Yang, H., Liang, N., Zhang, X., and Zhang, F. (2020). Are immune-related adverse events associated with the efficacy of immune checkpoint inhibitors in patients with cancer? A systematic review and meta-analysis. BMC Med. *18*, 87. https://doi.org/10.1186/s12916-020-01549-2.

124. Shalapour, S., and Karin, M. (2021). The neglected brothers come of age: B cells and cancer. Semin. Immunol. *52*, 101479. https://doi.org/10.1016/j.smim.2021.101479.

125. Roumenina, L.T., Daugan, M.V., Noé, R., Petitprez, F., Vano, Y.A., Sanchez-Salas, R., Becht, E., Meilleroux, J., Clec'h, B.L., Giraldo, N.A., et al. (2019). Tumor cells hijack macrophage-produced complement C1q to promote tumor growth. Cancer Immunol. Res. *7*, 1091–1105. https://doi.org/10.1158/2326-6066.CIR-18-0891.

126. Roumenina, L.T., Daugan, M.V., Petitprez, F., Sautès-Fridman, C., and Fridman, W.H. (2019). Context-dependent roles of complement in cancer. Nat. Rev. Cancer *19*, 698–715. https://doi.org/10.1038/s41568-019-0210-0.

127. Cui, C., Wang, J., Fagerberg, E., Chen, P.-M., Connolly, K.A., Damo, M., Cheung, J.F., Mao, T., Askari, A.S., Chen, S., et al. (2021). Neoantigen-driven B cell and CD4 T follicular helper cell collaboration promotes anti-tumor CD8 T cell responses. Cell *184*, 6101–6118.e13. https://doi.org/10.1016/j.cell.2021.11.007.

128. Rodriguez, A.B., Peske, J.D., Woods, A.N., Leick, K.M., Mauldin, I.S., Meneveau, M.O., Young, S.J., Lindsay, R.S., Melssen, M.M., Cyranowski, S., et al. (2021). Immune mechanisms orchestrate tertiary lymphoid structures in tumors via cancer-associated fibroblasts. Cell Rep. *36*, 109422. https://doi.org/10.1016/j.celrep.2021.109422.