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Host tissue determinants of tumour immunity

Helénè Salmon[1,2,3,*], **Romain Remark**[4], **Sacha Gnjatic**[1,2,5], **Miriam Merad**[1,2,*]

[1]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

[2]Precision Immunology Institute and Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

[3]INSERM U932, Institut Curie, Paris, France.

[4]Innate Pharma, Marseille, France.

[5]Department of Hematology and Oncology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Abstract

Although common evolutionary principles drive the growth of cancer cells regardless of the tissue of origin, the microenvironment in which tumours arise substantially differs across various organ sites. Recent studies have established that, in addition to cell-intrinsic effects, tumour growth regulation also depends on local cues driven by tissue environmental factors. In this Review, we discuss how tissue-specific determinants might influence tumour development and argue that unravelling the tissue-specific contribution to tumour immunity should help the development of precise immunotherapeutic strategies for patients with cancer.

Immunotherapy has led to remarkable recent achievements in cancer treatment with the induction of strong and durable clinical responses in cancers traditionally thought to have poor outcomes. In particular, blockade of the T cell inhibitory checkpoint molecules cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD1) — or its ligand, PD1 ligand 1 (PDL1) — can unleash antitumour T cell activity and lead to long-term clinical responses in patients with advanced cancer who had not responded to traditional therapies[1]. In the past decade, this has led to US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval of antibodies targeting CTLA4 and/or the PD1-PDL1 axis in melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC), Hodgkin lymphoma, bladder cancer, head and neck squamous cell carcinoma (HNSCC), Merkel cell carcinoma, microsatellite instability (MSI)-high tumours, gastro-

* helene.salmon@curie.fr; miriam.merad@mssm.edu.

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oesophageal cancer, hepatocellular carcinoma (HCC) and, more recently, cervical cancer and small-cell lung cancer (SCLC)[1–4].

However, the clinical benefit of immune checkpoint therapies remains limited to a subset of patients, with overall response rates varying widely across cancer types. Patients with pancreatic cancer, prostate cancer or colorectal cancer (CRC) lesions rarely benefit from current immune checkpoint blockade regimens, reflecting either tumour cell-inherent resistance mechanisms or extrinsic factors restraining antitumour immunity. Uncovering the mechanisms of response and resistance to PD1 immunotherapy is at the forefront of cancer research. One of the most established tumour factors associated with increased response to immune checkpoint blockade is the presence of high tumour mutational burden (TMB)[5–8]. High TMB is thought to increase the occurrence of mutation-derived tumour-specific epitopes (also called neoantigens), thereby promoting immune recognition of tumour cells and leading to enhanced therapeutic activity[9].

While tumour-intrinsic features have been shown to affect immune responses across cancer types (as reviewed elsewhere[10]), emerging evidence suggests a possible contribution of host tissue determinants in shaping tumour immunity. For example, first, cancers that are poorly responsive to PD1 blockade, such as ovarian or prostate cancers, can still retain a substantial mutational load[11], suggesting that adaptive immunity to tumours is not determined by TMB alone. Second, unfavourable gut microbiome signatures[12,13] are associated with poor tumour response to immune checkpoint blockade, highlighting the role of tumour-extrinsic factors in controlling cancer response to immunotherapy. Third, although T cell infiltration in tumour lesions correlates with improved tumour response to immune checkpoint blockade, the presence of T cells in ovarian[14] and colorectal[15] cancers does not correlate with response to immunotherapy, suggesting that additional features of these tissues may contribute to tumour outcome. Fourth, studies of ‘seeds’ (cancer cells) versus ‘soil’ (the invaded host organ) have revealed that the immune contexture can strongly differ between primary and metastatic tumour lesions[16,17]. Accordingly, in mice, the same melanoma cells implanted in the skin or the lung tissue have distinct tumour-associated macrophage (TAM) infiltrates[18], supporting a role of the soil in regulating antitumour responses.

The tumour microenvironment (TME) is composed of a heterogeneous mixture of tissue-resident immune cells, fibroblasts, endothelial cells (ECs) and neurons that predate tumour formation, together with blood-derived cells that are recruited to the tumour site upon cancer progression[19]. Each of these cellular components can be co-opted by the tumour and contribute to cancer progression[20]. However, there are major differences in the composition and spatial organization of the tissue microenvironment across cancer types, including differences in tissue vascularization and innervation and tissue-resident immune and stromal cellular networks that may impact local immunosurveillance and the induction of tumour immunity regardless of the inherent tumour immunogenicity (FIG. 1).

In this Review, we provide an overview of recent insights into the role of host tissue cellular and molecular cues in regulating local immunity and of how these tissue determinants may influence the antitumour immune response across disease sites. We also discuss potential implications for future research and clinical therapeutic strategies.

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Tissue-specific immune microenvironment

Distinct features of tumour immune cell infiltration across tumour types.

Immune cells form a major cellular compartment of tumour lesions. Although in most cancer types, the immune microenvironment is often dominated by monocytes and/or macrophages and T lymphocytes[21–27] accumulating at the invasive margin and in the peritumoural stroma[8,28,29], the cellular composition and functional state of tumour-infiltrating immune cells vary considerably across tumours.

Transcriptional analysis of 10,000 tumours comprising 33 different cancer types performed by The Cancer Genome Atlas (TCGA) consortium identified several immune expression signatures that interestingly spanned anatomical location yet substantially varied in their proportion across cancers, with some immune signature modules dominating specific tumour types[30]. Uveal melanoma and brain tumours have the lowest immune cell infiltration, which is strongly dominated by macrophages over lymphocytes and natural killer (NK) cells[24,30,31], which likely contributes to their limited response to immune checkpoint blockade[32]. Conversely, tumours containing the highest immune cell fraction include malignancies most responsive to immunotherapy, such as lung carcinoma, HNSCC and skin melanoma[30]. Mass cytometry and single-cell RNA sequencing analyses of these tumours have revealed a high frequency of T cells in the immune compartment, often dominating the myeloid cell pool[26,33,34]. Pancreatic cancer lesions contain a strong immune infiltrate frequently dominated by macrophages[35]. The myeloid-inflamed stroma in pancreatic ductal adenocarcinoma (PDAC) has been shown to be associated with restricted T cell functionality in both human and mouse lesions[22,36], which may contribute to the poor clinical responses to immunotherapy observed in patients with PDAC2,22,37 (Fig. 1).

The strong variations in lymphocytic infiltration across cancers may be driven by the distinct molecular compositions and states of antigen-presenting cells (APCs) found between tissues. Non-lymphoid tissue APCs present in sterile tissues such as the pancreas and brain, filtering sites such as the kidney and liver, and environmental interfaces such as the skin, lung and gut differ from each other38. Those distinct locations expose APCs to different levels of signals from microorganisms or proliferating cells (for example, the epithelium), which can imprint their composition and immune function on an organ site, which in turn likely shapes tumour T cell infiltrates in tumour lesions.

Tertiary lymphoid structures across cancer types.

Similar to most chronically inflamed lesions, tumour lesions frequently display ectopic lymphoid-like structures called tertiary lymphoid structures (TLSs), preferentially found at the invasive margin, that contain large numbers of B cells, $CD4^+$ T cells and mature dendritic cells (DCs)[39–44]. More than 60% of NSCLC, CRC, PDAC, ovarian cancer and breast cancer lesions and more than 45% of HCCs contain TLSs, whereas RCCs, uveal melanomas and brain tumours are almost devoid of such structures[45–48]. Interestingly, the B16-OVA melanoma cell line, when injected either subcutaneously or intraperitoneally in mice, induced TLS formation around peripheral node addressin $(PNAd)^+$ vasculature in all intraperitoneal tumours but not in subcutaneous tumours[49], supporting the idea that the local

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environment of the skin poorly supports TLS formation. However, in human melanoma resection samples, approximately 10–30% of primary lesions or cutaneous metastases contain TLSs[40,50,51]. Thus, it is currently unclear whether the local environment of the skin is poorly permissive to TLS development[40,49,52] and/or whether other intrinsic host tissue factors might be involved.

The molecular mechanism s leading to lymphoid neogenesis are only partially understood53. They appear to be similar to those that lead to the development of new secondary lymphoid organs (SLOs), which are orchestrated by haematopoietic cells (the lymphoid tissue inducer (LTi) cells) and stromal cells (the lymphoid tissue organizer (LTo) cells), which differentiate into follicular dendritic cells and fibroblastic reticular cells[54].

TLS neogenesis is driven by inflammatory molecules and cell types that may differ across tumour tissues. Similar to SLOs, immune cells, fibroblasts and lymphotoxin-α (LTα) and LTβ signalling are required for TLS form ation[53]. Several studies have reported that resident stromal cells can become LTo cells under inflammatory conditions and are able to initiate TLS form ation[55], whereas activated lymphocytes substitute for LTi cells[56]. Cytokines (such as interleukin-17 (IL-17), IL-22, IL-23 and IL-36), chemokines (such as CXC-chemokine ligand 12 (CXCL12), CXCL13, CC-chemokine ligand 19 (CCL19) and CCL21), adhesion molecules (such as mucosal addressin cell adhesion molecule 1 (MAdCAM1), vascular cell adhesion molecule 1 (VCAM1) and intercellular cell adhesion molecule 1 (ICAM1)) and high endothelial venule neogenesis seem to play an important role in TLS development[49,57,58]. Conversely, regulatory T (T_{reg}) cells negatively regulate TLS neogenesis[59,60].

Thus, differences in stromal cells, immune infiltration and molecular composition at the tissue site could dramatically influence TLS formation. In the lung, TLSs called bronchus-associated lymphoid tissues develop spontaneously in multiple conditions of infection, cancer and chronic inflammation in both mice and humans61–64. A recent mouse study showed that type I interferons induce platelet-derived growth factor-α (PDGFRa)$^+$ lung fibroblasts to secrete CXCL13 upon influenza virus infection, leading to B cell recruitment and germinal centre formation[65]. The strong ability of lung tissue stromal cells to respond to inflammatory mediators could contribute to the high frequency of TLSs observed in tumour lesions from patients with lung cancer[41,64]. The reduced stromal compartment and lack of pro-TLS-forming chemokines and cytokines may explain the reduced TLS number in RCC[53] and brain tumour lesions — both glioblastoma and lower-grade glioma[17]. Further investigation is needed to better understand the different TLS frequencies observed across anatomical sites.

The tumour immune microenvironment: impact on clinical outcomes.

The impact of the immune infiltrate on patient survival also differs between tumour types. A worldwide task force including 14 different centres recently validated the benefit of CD3$^+$ and CD8$^+$ T cell quantification in tumours (Immunoscore) to stratify prognosis of patients with colon cancer independently of the tumour-node-metastasis (TNM) stage[66]. In melanoma, HNSCC, NSCLC and breast, bladder, urothelial and ovarian cancers, high densities of tumour-infiltrating cytotoxic and memory T cells and skewing towards a T

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helper 1 (T_H1) cell immune response are also associated with improved overall survival and disease-free survival[47]. Surprisingly, in RCC and prostate cancer, high $CD8^+$ T cell densities are associated with poor overall survival[15,67]. In RCC, the adverse microenvironment characterized by an increased level of angiogenesis, immature DCs, T_{reg} cells and high PDL1 expression might explain this negative association with prognosis[68]. In line with this, $CD8^+$ T cells purified from tumour lesions of patients with RCC have been shown to be polyclonal and poorly cytotoxic and display phenotypic profiles similar to the poorly activated adjacent kidney-infiltrating T cells[69].

Tissue-resident bona-fide DCs are the most immunogenic APCs and are uniquely able to prime efficient effector immune responses against tumour cells. Cross-presenting tissue-resident DCs (also called DC1s, which are characterized in mice by the expression of the integrin CD103) excel at driving antitumour $CD8^+$ T cell immunity[70–72]. DC1s are required for tumour response to PD1-PDL1 blockade, as shown in mouse melanoma tumours[71,73]. Importantly, mice lacking DC1s have reduced infiltration of endogenous $CD8^+$ interferon-γ $(IFN\gamma)^+$ T cells in lung cancer lesions (M.M., unpublished observations). While reduced tumour T cell infiltration in DC1-deficient animals is likely due to defective priming in the draining lymph node, in vivo imaging studies revealed that proliferating $CD8^+$ T cells were closely interacting with DC1s in the TME[74]. These results suggest that, in addition to their role in the draining lymph node, DC1s may also contribute to the reactivation and expansion of T cells in the TME. Given that the presence of tumour-infiltrating T cells correlates with tumour response to immune checkpoint blockade[75,76], densities of tumour-infiltrating DC1s may provide a potential biomarker of tumour response to immune checkpoint blockade[77]. Their abundance has been shown to predict responses to anti-PD1 therapy in human metastatic melanoma[78]. Interestingly, we found that lung tissue is one of the most enriched organs for cross-presenting DCs (M.M., unpublished observations), which may contribute to shaping lung tumour T cell content at baseline.

The accumulation of immune cells positive for CD68 — a pan-macrophage marker — is associated with increased survival in colorectal or prostate cancers but poor clinical outcome in melanoma, RCC, HNSCC and breast, bladder and pancreatic cancers[47]. These contradictory prognoses may result from the imprecision of this marker — which labels a wide diversity of macrophages in addition to DCs — as well as differences in origin, activation levels, response to stimuli[79,80] or specific functional attributes that are deleterious to some tumour lesions but not all. For example, the surfactant clearance function of alveolar macrophages may impact their metabolic ability and potentially their immunogenicity. Better characterization of macrophage populations — beyond the simple expression of CD68 or CD163 markers — through the use of high-dimensional profiling is critically needed to foster our understanding of the role of TAMs in cancer progression.

NK cell infiltration correlates with increased overall survival in clear-cell RCC[81], CRC[82] and melanoma[78]. In addition to direct NK cell antitumour effects — through secretion of perforin and granzyme and expression of tumour necrosis factor (TNF)-related apoptosis-inducing ligand receptor (TRAILR; also known as TNFRSF10)[83–85] — the antitumour impact of NK cells has also been attributed to their secretion of the chemokines CCL5 and XCL1 and the DC growth factor FMS-like tyrosine kinase 3 ligand (FLT3L), which regulate

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tumour DC recruitment and abundance, as recently shown in mouse melanoma lesions[78,86]. Conversely, the infiltration of NK cells has no impact on clinical outcome in NSCLC, where their cytotoxic functions have been shown to be impaired by a strong reduction in NK cell activating receptors including NKp30, NKp80 and DNAM1 (REF[27]). This altered phenotype was specifically observed in the tumour and not the distant lung tissue, indicating that the NSCLC TME locally impairs NK cells[27]. Of note, NK cells were found in only the peritumoural stroma of human NSCLC sam ples[27,87], whereas they were observed throughout the tumour in RCC, emphasizing the need to consider immune cell spatial distribution in addition to global infiltration in tumour lesions[88]. Interestingly, some tumour types such as HNSCC are strongly infiltrated by NK cells and may benefit from NK cell-targeting immunotherapies, as recently shown with an NKG2A-blocking antibody promoting the antitumour activity of both NK cells and $CD8^+$ T cells[89].

Stromal cell contribution to cancer immunity

Most solid tumours comprise two distinct compartments that include the tumour parenchyma and a surrounding stroma. Depending on the cellular origin of the malignant cell — epithelial, melanocyte or mesenchymal — the tumour mass differs in its organization, cellular cohesion and tumour-stroma boundaries, which impacts on immune cell distribution and function (BOX 1). This section primarily focuses on the nonhaematopoietic stromal compartment, a connective tissue comprising fibroblasts, pericytes and lymphatic and blood vessels as well as nerve cells all embedded in extracellular matrix (ECM) components such as collagen and fibronectin fibres[90]. Beyond immune cells, the dominant cellular components of the tumour stroma are blood ECs and cancer-associated fibroblasts (CAFs), both of which have been shown to strongly influence the antitumour immune response. Nerve cells, the microbiota and tissue-resident macrophages (Tr-macs) are also discussed as host tissue cellular components impacting tumour immunity (FIG. 2).

Tissue-specific vascular features.

The vascular system is highly specialized and adapted to the needs of different organs, with differences in cohesiveness, pericyte coverage and expression of adhesion molecules (for example, P-selectin and ICAM1), all of which regulate vessel permeability and immune cell extravasation. For example, the blood vessels of the liver are lined by discontinuous ECs favouring cellular trafficking, whereas the retina and brain endothelia are characterized by numerous tight junctions linking their ECs and limiting immune cell ingress91. Accordingly, ECs isolated from various anatomic sites present distinct gene expression profiles[92–94].

In the tumour context, new blood vessels — that primarily sprout from the host organ vasculature[95] — are generally altered and characterized by structural abnormalities and increased permeability[96]. However, several lines of evidence support the presence of tissue-specific vascular features in the TME. The tumour vasculature is sparse and leaky in PDAC lesions, which is thought to be primarily explained by the extensive deposits of ECM molecules in the TM E resulting in interstitial hypertension and vascular compression[97] (FIG. 1). By contrast, glioblastomas and RCCs are characterized by high microvascular

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proliferation[68,98] (FIG. 1). In line with the important contribution of host tissue determinants to site-specific tumour vascular features, multiple studies have shown that the tumour vasculature differs substantially between similar tumours growing in different host organs[99,100]. For instance, human RCC xenografts implanted subcutaneously into nude mice displayed low numbers of vessels, whereas the same tumours injected into the kidney were highly vascularized[101]. This can be attributed to non-tumour-cell-derived local microenvironment signals, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) by fibroblasts or macrophages[101,102], as well as intrinsic site-specific properties of ECs[91,92]. ECs from distinct tissues exhibit different responses to tissue damage and inflammatory signals[93,103], partly owing to the differential expression of pattern recognition receptors as well as receptors for cytokines and growth factors (for example, TNF receptor (TNFR) and VEGF receptor (VEGFR))[93,104], which contributes to differences between EC phenotypes across cancers. A mouse study showed that RCC metastases to the liver are more reliant on VEGFR1 to induce angiogenesis than the same metastases to the lung owing to differential activity of VEGFRs on liver versus lung ECs[105].

The tumour vasculature contributes to regulating the composition of the immune infiltrate through structural features that control immune cell extravasation and the homing of specific immune cell subsets. For example, hepatic sinusoidal ECs in the inflamed human liver and HCC express high levels of the receptor common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER1; also known as stabilin 1), as well as vascular adhesion protein 1 (VAP1), which have been shown in vitro to favour the selective extravasation of T_{reg} cells[106]. A similar observation was made with PDAC ECs through the expression of another combination of addressins, including E-selectin and MAdCAM1 (REF[107]). In vivo studies will be needed to further investigate whether these distinct molecules provide organ-specific signals for T_{reg} cell recruitment to HCC and PDAC lesions. Tumour ECs also shape the tumour immune infiltrate through direct immunosuppression by expressing immunomodulatory molecules such as PDL1 (REF[108]) or FAS ligand (FASL)[90,109]. A study analysing tissue microarrays of more than 600 samples of human renal, breast, ovarian, colon, bladder and prostate cancers detected expression of FASL on tumour ECs predominantly in CRC and ovarian cancer lesions, with less frequent expression on RCC and breast tumour ECs[109]. Further mechanistic analysis showed that FASL-expressing ECs from human ovarian tumours favour immune tolerance by killing effector T cells[109].

Cancer-associated fibroblast heterogeneity across tumour types.

Fibroblasts are mesenchymal cells that play a critical role in shaping the structure of organs in homeostasis, through synthesis and remodelling of the tissue ECM[110], and in secreting cytokines, chemokines and growth factors, thereby maintaining the homeostasis of adjacent cells and orchestrating the inflammatory response upon tissue damage. Although bone marrow mesenchymal cells contribute to the generation of CAFs in mice[111–113], most studies including analysis of bone marrow chimaeras and parabiosis experiments have shown that CAFs derive mainly from local stromal precursors[90,112,114,115]. Interestingly, transcriptome analyses have emphasized the heterogeneity of the fibroblast network across organs at baseline[116–119] and revealed tissue-specific fibroblast programmes, including transcription factors, guidance molecules and growth factors, as well as ECM components

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and enzymes for ECM remodelling[116,118,119]. Accordingly, a recent proteomics study that compared ECM molecules from 11 porcine tissues including skin, lung, pancreas and brain revealed major differences in matrix composition and relative abundance across tissue types[120].

Beyond the ability of CAFs to modulate tumour cells directly, CAFs also control tumour progression through regulation of tumour immunity[90]. CXCL12 has been shown to be expressed by CAFs in a broad range of tumour tissues yet linked to different functions, that is, T_{reg} cell attraction as shown in vitro with primary human breast cancer cells[121] and T cell exclusion as shown in mouse pancreatic and lung tumour models[122]. The abundance of CAFs also underlies strong stromal differences across cancers. Breast, colorectal, gastric and pancreatic tumour lesions generally contain a CAF-rich stroma, whereas fibroblasts are found in lower numbers in RCC and brain tumours[31,121,123–125]. Given the key role of fibroblasts in producing ECM molecules in the TME, the relative CAF abundance can have a strong impact on the immune infiltrate across tumour sites. The stroma represents a high fraction of the tumour mass in desmoplastic cancers such as PDAC, where the abundant CAFs secrete high amounts of ECM molecules, creating a physical barrier of dense fibres that limit immune cell infiltration[126]. Dynamic imaging of T cells deposited onto fresh human lung tumour slices revealed that in addition to their density, the orientation of stromal fibres parallel to the tumour-stroma boundary controls T cell motility and restrains them from contacting cancer cells[29] (FIG. 1).

Compared with solid tumours, haematological tumour lesions localized in the bone marrow or lymph nodes are often less fibrotic, and their looser architecture is thought to facilitate immune cell dynamics and their interactions with neoplastic cells[127]. A better understanding of CAF regulation of T cell access to tumour sites across cancers is crucial to improve chimeric antigen receptor (CAR) T cell accumulation in solid tumours and to increase the accumulation of endogenous tumour-specific T cells in cancer lesions, which correlates with increased response to immune checkpoint inhibition[8,75,128].

We are just starting to unravel the heterogeneity of the CAF compartment[33,115,121,129], and further investigation is needed to better decipher CAF differences between tumour types. Several studies in animal models have started to provide insight into the contribution of both tissue and tumour cues in shaping the CAF compartment[126,130–133]. Data comparing CAFs in skin and cervical tumours driven by the same oncogene showed that despite similarities in oncogene drivers, CAF transcriptional identity differed between the two tissue sites[130]. Skin CAFs expressed a pro-inflammatory gene signature including genes encoding the two cytokines IL-1β and IL-6 and two chemoattractants for neutrophils and macrophages, CXCL1 and CXCL2, whereas cervical CAFs did not, supporting an important role for the host tissue in regulating CAF phenotype. Accordingly, a comprehensive study of ECM proteins in mouse primary breast tumours and at two metastatic sites — lung and lymph node — showed organ-specific changes in matrix composition[131]. Importantly, tissue-specific matrix differences have been shown to impact immune cell behaviour, including T cell motility as well as macrophage morphology and polarization[29,120,134]. However, studies have also revealed similarities in the fibrotic stroma of primary and metastatic lesions of PDAC[126,133], which in addition to a direct impact of cancer cells on the host stroma may

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partly be explained by the fact that tumour cells might travel with stromal cells — bringing their own 'soil' — to the metastatic tissue[135]. Altogether, these data support the dual contribution of both tumour-intrinsic features and external cues mediated by the local environment in shaping the CAF compartment and hence the stromal architecture and immunomodulatory functions across tumour types (FiG. 1).

Other host factors shaping tumour tissue immune cell infiltrates.

The presence of nerves within tumour lesions has been reported in various cancer types, particularly in prostate[136], gastrointestinal[137,138] and pancreatic[139] cancer, with the density of nerve fibres generally associated with poor clinical outcomes[136]. Several studies have attributed the pro-tumoural effect of innervation to direct neuronal stimulation of tumour cell survival, proliferation and dissemination through release of neurotransmitters such as catecholamines and acetylcholine[138–141]. More recently, adrenergic nerve-derived noradrenaline has been shown to promote tumour-associated angiogenesis by regulating oxidative metabolism in tumour ECs[142]. While poorly studied in the TME, nerve-immune cell interactions are well described in healthy and inflamed tissues[143], in particular, the crosstalk between neurons and macrophages in gut tissue[143–145], where neurotransmitters mainly exert immunosuppressive functions[144,146]. Mouse studies suggest that targeting nerve fibres or neurotrophic factors may prove useful in the treatment of highly innervated cancer lesions[138,139,147]. In support of this, it has been suggested that β-blockers, traditionally used for cardiovascular disorders, could increase the survival of patients with cancer, as observed for patients with PDAC and prostate tumours[139,148]. More studies are needed to investigate the contribution of nerve-immune cell interactions to these effects of β-blockers and to identify novel specific components of the neuro-immune crosstalk to target.

Whereas immune cells in tissues are typically thought to derive from adult haematopoiesis in the bone marrow and be recruited into tissues mostly during tissue injuries, recent studies have revealed that some immune cell populations can remain in tissues for very prolonged periods of time and contribute to tissue homeostasis and integrity independently of blood circulation[80] (BOX 2). The most striking example of tissue-resident immune cells are Tr-macs. Most Tr-macs arise from embryonic precursors that are recruited to tissues before birth and are self-renewing independent of adult haematopoiesis[149–153]. Consistent with their life cycle, Tr-macs are heavily imprinted by tissue-specific cues and express unique transcriptional and enhancer programmes depending on the tissues in which they reside[79]. In addition to their role in tissue immunity, Tr-macs play a key role in tissue homeostasis. For example, in the gut, Tr-macs respond to microbial cues to enhance T_{reg} cell function[154] and to promote intestinal transit through the regulation of enteric neuronal function[145]. Most tumours produce myeloid cytokines that contribute to the recruitment of circulating monocytes and their differentiation into macrophage-like cells. Although monocyte-derived macrophages and Tr-macs share some phenotypic markers, they have a distinct molecular programme and likely play distinct roles in tumour outcome[80]. Fate mapping studies from our group and others revealed that TAMs result from a mixture of the two macrophage lineages (Tr-macs that precede tumour formation and monocyte-derived TAMs that are recruited to the tissue upon tumour progression)[155] (M.M, unpublished observations). These two TAM lineages likely contribute differently to tumour outcome. For example, as we

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recently showed in breast cancer lesions, Tr-macs shape early tumour progression and early metastasis[156]. Fate mapping studies of macrophages in PDAC lesions revealed that embryonically derived Tr-macs exhibited a unique pro-fibrotic transcriptional profile distinct from that of their bone marrow-derived counterparts, which instead exhibited a programme enriched in antigen presentation[155]. However, the development of TAM-specific targets has proved extremely difficult. This is in large part because we still do not have a complete understanding of the functional diversity of the TAM compartment. This is particularly true for human macrophages, whose biology has primarily been studied using human monocyte-derived macrophages generated in vitro, with limited knowledge of human Tr-macs. We believe that understanding the functional programme of Tr-macs in each type of tumour lesion may help unravel the tissue-specific influence on tumour outcome.

Recent reports suggest the potential key contribution of the gut microbiome to the modulation of antitumour immunity[12,13,157]. In these studies, the gut microbiome is thought to modulate tumour immunity through its systemic effect on the immune system[158,159]. Multiple anatomic sites beyond the gastrointestinal tract are in close association with commensal bacteria. Analyses of microbiota from the gut, skin, lung, vagina and oral cavity have revealed site-specific commensal communities that shape the tissue immune tone[160] by acting as local adjuvants setting the threshold of effector cell activation. Several studies demonstrate the role of the local tissue microbiome: in the skin, commensals shape T cell function independently of the gut microorganism s[161], and the lung tissue microbiota contributes to respiratory health[162], whereas gut microorganisms contribute to T_{reg} cell function and intestinal peristalsis[145]. A recent mouse study shed light on the role of the local microbiota in shaping tumour-associated immune responses, showing that the lung microbiome promotes lung cancer development by stimulating IL-1β and IL-23 production from myeloid cells, which in turn activate lung-resident γδ T cells[163]. Thus, in addition to studies of the gut microbiota, further analysis of the local microbiome and its dysbiosis especially in interface tissues such as skin and lung may uncover novel immunoregulatory pathways at the tumour site.

Therapeutic implications

With the growing list of US FDA approvals for antibodies targeting immune checkpoints, it would be tempting from a therapeutic standpoint to dismiss differences described above in the immune microenvironments of these various tissues as minor. However, while revolutionizing cancer treatment, these novel immunotherapies work in only a subset of patients, and overall response rates vary widely on the basis of tissue and stage, suggesting that, while there is some intrinsic tumour susceptibility to the mechanisms of action of these drugs, response is also based on lymphocytic infiltration and presence of immunomodulatory cells or molecules, among some of the commonly proposed biomarkers predicting response[8,164]. Moreover, whether indolent or aggressive, prevalent diseases like hormone-sensitive breast cancer, prostate cancer or microsatellite-stable (MSS) CRC rarely appear to benefit from current immune checkpoint blockade regimens[165,166]. Therefore, to maximize the impact of novel immunotherapies as well as conventional treatments on patient care, researchers in the field have honed their efforts on biomarker discovery, in particular, on tissue-based immune monitoring that encompasses immunopheno-typing,

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functional immune assays, immunogenomics and immunopathology. FIGURE 3 summarizes the tissue specificity of immune, stromal and tumour components and mechanisms and their potential therapeutic implications.

Improving the accessibility of tissues to increase immunotherapeutic efficacy.

One line of evidence to explain differential immunotherapeutic potential across different solid tumours may simply reside in immune cell accessibility to tissues. For tumour types with relatively poorer lymphocytic infiltration, such as glioblastoma, or for cancers where vast differences are seen from patient to patient, an attractive approach to improve therapeutic effectiveness is to alter the tumour capacity for immune infiltration, in particular, to convert so-called immune deserts or T cell-excluded tumours into highly infiltrated ones. So far, cell-based therapies such as CAR T cells have met the greatest success in haematological malignancies (acute lymphocytic leukaemia, diffuse large B cell lymphoma and multiple myeloma), likely owing to the loosely structured environment of the bone marrow and blood that may help maximize interactions between effectors and their targets (in addition to the prevalence of tissue-restricted surface antigens such as CD19 or B cell maturation antigen (BCMA; also known as TNFRSF17) targeted by these treatments). By contrast, compartmentalized compact solid tumours have not fared as well with adoptive T cell transfer (ACT) alone[167,168]. In addition to a paucity of truly tumour-specific surface antigens to target, it is likely that transferred effectors may fail to penetrate the tumour either owing to tight stromal packaging and limited vascularization physically limiting ingress or owing to tissue-resident immunomodulators, such as dysregulated chemokine production from myeloid cells or active suppression by T_{reg} cells. Responsiveness to immune checkpoint blockade, which ultimately relies on adaptive immunity locally at the tumour site, provides hope that ACT may eventually work in solid malignancies if the transferred T cells can navigate their way in sufficient numbers to the tumour site and if appropriate antigens can be safely targeted.

Strategies have been explored to bypass the stromal cell and ECM barrier in CAF-rich solid tumours. Unexpectedly, CAF depletion promoted tumour progression in two studies using mouse models of pancreatic cancer, which was attributed to increased angiogenesis and T_{reg} cell infiltration[169,170]. More encouraging are strategies to reduce stromal ECM[97,126,134] or reprogramme CAFs[8,132]. Promising results were obtained in preclinical studies with drugs targeting matrix molecules, such as enzymatic agents degrading hyaluronan[97,126], or CAR T cells expressing the enzyme heparanase, which degrades heparan sulfate proteoglycans, major ECM com ponents[134]. Lack of response to immune checkpoint blockade has been associated with a transforming growth factor-β (TGFβ) signature in CAFs of several human tumour types including bladder cancer and melanoma[8,171], and blocking TGFβ in mouse models of breast cancer increased T cell penetration into the tumour parenchyma, improving tumour response to PDL1 blockade[8]. A challenge for future development of these types of therapy is to better understand CAF biology, in particular, features and gene expression signatures that distinguish tumour-associated fibroblasts from normal fibroblasts, to specifically target CAFs and minimize adverse events[172]. Of note, preconditioning strategies that include total body irradiation or preparative chemotherapy regimens have improved clinical efficacy of ACT[173]. In addition to expansion of transferred cells in the

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lymphodepleted host and reduction in T_{reg} cells, alteration of the tissue architecture might also contribute to the effect by maximizing T cell infiltration at the tumour site.

Tissue-specific antigenic profiles and response to immune checkpoint blockade.

Antigens known to be recognized by immune effectors include those that are uniquely specific to individual tumours (random passenger mutation-derived neoantigens), those that are shared across various tumour types but distinct from normal tissue (cancer testis, stem cell, endogenous retroviral and oncofetal antigens), those that are broadly expressed tissue-specific targets (differentiation antigens) and those with viral aetiology (for example, human papillomavirus and hepatitis C virus). Each category has proved successful in acting as rejection antigens from case reports of patients with established cancers following ACT and vaccine strategies, thereby establishing proof-of-principle that many different tumour antigens are valid to pursue. The likelihood of a mutation generating a neoepitope increases with a higher number of mutations, and tumours with a carcinogen aetiology (such as ultraviolet light exposure in melanoma and smoking in lung and bladder cancer) are also among the most responsive to immune checkpoint blockade therapy[5–7].

Antigenic content could therefore be partially responsible for the differential response to immune checkpoint blockade, with evidence that colon, breast and prostate tumours have less expression of cancer testis antigens than do melanoma, lung or ovarian tumours[174]. Furthermore, many common cancers where PD1 intervention is futile do not have a lesser mutational burden and fewer potential neoepitopes, as these are within the range of numbers seen in approved tumour types such as bladder and renal cancer or HCC. Tissue-driven antigen content and resulting immunogenicity based on the cell of origin may therefore be more important predictors of lack of response to immune checkpoints than simply the number of mutations. In support of this, a recent paper indicates that MAGEA antigens are negative predictors of CTLA4 but not PD1 response in patients with melanoma independent of TMB[175]. The underlying mechanism suggested in this study is MAGEA protein-associated suppression of autophagy, a process known to induce potent immune responses by cytokine-mediated priming of effector T cells[176]. Thus, tumour antigen content and composition will need to be treated in a tissue-specific manner to maximize the rational design of immune-mediated treatments.

Implications of tissue-specific tumour immunity for combination therapies.

The presence of immune effectors in tumours should logically be dependent on antigenicity, as a tumour cell-intrinsic characteristic. Nevertheless, evidence is accumulating that immune infiltration and antigenicity are not always correlated, with similar predicted epitope frequency seen in somatic mutations, differentiation antigens and cancer testis antigens in inflamed and non-inflamed tumours, such as melanoma or myeloma[177–179]. In colon cancer, a high Immunoscore is of good prognostic value in tumours with both high and low mutational burden, even if patients with MSI-high disease generally have more immune infiltration[180]. Despite having tumour-infiltrating lymphocytes, adequate antigenicity and susceptible pathways, tumours where PD1 treatment is unsuccessful either fail to have enough of these factors occurring simultaneously or have other dominant characteristics preventing them from responding more frequently. These could either be driven by the

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tumour itself, such as intrinsic mutations leading to defects or losses in the antigen presentation machinery or in the capacity to attract cross-presenting DCs, or be related to non-tumour idiosyncrasies in the tissue of origin. For example, hormone addiction in prostate and breast tumours driving oncogenic growth[181], mucinous layers preventing physical access of T cells in ovarian cancer[182] and/or microbiome interactions thwarting proper anti-tumour immune priming in CRC[183] should be further explored as potential confounders for the lack of efficacy of immune checkpoint inhibitors.

Patients resistant to or relapsing after immune checkpoint blockade may still benefit from tissue-specific modulation strategies. Targeting of Tr-macs with monoclonal antibodies or small molecules to chemokine receptors or cytokines[184] is a promising avenue that could eventually have a clinical impact on tumours in organs with specific myeloid cell prevalence or unique characteristics, such as microglia for glioblastoma or brain metastases.

As discussed earlier in this Review, the combination of EC-intrinsic features and the integration of signals from tumour cells and the TME triggers immunomodulatory pathways in ECs that shape the outcome of anti-tumour immune responses differently across cancer types, which should be considered for the development of therapeutic strategies. RCC, characterized by aberrant angiogenic signalling and a strongly immune tolerogenic environment, could particularly benefit from the combination of vessel modulation and immune checkpoint blockade, as suggested by recent clinical trials[185].

Immunotherapies based on T cell activation should in theory be tumour agnostic because they do not directly target the tumour itself. Therefore, even in hormone-sensitive tumours and MSS colon cancer, which rarely benefit from current immune checkpoint blockade regimens, it may be that the right combination of predictive biomarkers is required to select those very rare patients most likely to benefit. The corollary of this hypothesis is that tailored combinatorial interventions that modulate or induce these predictive biomarkers could rescue the lack of activity with immune checkpoints alone in the remaining patients.

In a given tumour, cancer therapies such as chemotherapy[186,187], radiotherapy[188] or immunotherapy[189,190] can remodel the immune contexture by acting directly or indirectly on different components of the tumour ecosystem such as cancer cells, immune or stromal cells or tumour vasculature. Therefore, a global evaluation of inter-patient (between and within given tumour types) and intratumour (spatial) variability will be needed to optimize the choice of interventional approach. Ultimately, therapeutic targeting of solid tumours is most likely to benefit from a biomarker-based combinatorial but tailored use of strategies to drive immune infiltration (targeting tumour-associated fibroblasts or vessels), prevent immunosuppressive mechanisms (immunomodulators) and minimize immune escape (multivalent vaccines).

Conclusions and future perspectives

In this Review, we have presented evidence that the strength of antitumour immunity is influenced not only by the tumour genetics but also by the tissue in which the tumour evolves. Most of our understanding of the human TME stems from low-throughput tissue

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imaging using immunohistochemistry, immunofluorescence imaging or transcriptional profiling of tumour lesions in bulk (such as TCGA). These methods are unable to capture the complexity of the tumour immune infiltrates, which consist of dozens of cell subtypes whose activation states are highly dependent on tissue location, environmental triggers and host genetics. Recent advances in high-throughput single-cell sequencing and cell profiling technologies are now enabling the cellular and molecular complexity of tumour lesions to be deciphered with unprecedented granularity. This Review emphasizes the need for building comprehensive tumour cell atlases for each cancer type and site at diagnosis, during treatment and upon relapse. The challenge for the near future is to stratify patients on the basis of the molecular and cellular states of the tumour cells and their cellular microenvironment. Deep and dynamic knowledge of the cellular network that constitutes tumour lesions beyond the tumour cell should help inform the mechanisms of resistance to treatment and transform the rational design of combination therapy trials.

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Box 1 |

Heterogeneity of the tumour architecture

Epithelial tissue, which lines all body cavities, is a laterally extending layer of connected cells resting on a basement membrane, with apical-basal polarity that separates the organ from the external environment. Carcinomas — cancers that derive from the epithelium — are commonly organized as multiple distinct islets of tumour cells. Within carcinomas, a basal lamina can separate the tumour mass from its surrounding tissue, although it is often disrupted after tumour cells gain invasive capabilities[191,192]. Before undergoing dedifferentiation and, in some cases, epithelial-to-mesenchymal transition, carcinoma cells often maintain expression of cell-cell junctions such as E-cadherin and tight junctions, which provide cellular cohesion to the tumour nests[193–195]. This cohesive cellular structure likely constitutes a physical barrier that limits the entry of immune cells such as lymphocytes and dendritic cells, which do not use protease degradation for their migration in tissues and must physically migrate through intercellular spaces[29,196].

In contrast to other solid tumours, carcinomas often overexpress mucins, which are transmembrane or secreted glycosylated proteins produced by epithelial cells[182]. In addition to protecting interface tissues against pathogens, mucins also restrict the activation of potentially damaging inflammatory responses at the epithelial barrier[182,197]. In tumours, mucins have been shown to mask surface antigens on target tumour cells, limiting antibody binding[198] and tumour cell killing by cytotoxic lymphocytes[199]. Of note, the transmembrane mucin MUC1 is also aberrantly expressed on malignant haematopoietic cells[200], suggesting that other cancers could also exploit the immunoregulatory function of mucins to promote growth.

A different architecture is observed in non-carcinoma tumours such as glioblastoma, the most common malignant primary brain cancer lesion in adults, and is characterized by lower cell cohesion and poorly defined tumour margins[201]. In melanoma, it is also not uncommon to observe small clusters or individual tumour cells interspersed with extracellular matrix molecules in the tumour mass, a feature that is particularly striking in desmoplastic melanoma, which, together with a high tumour mutational burden, may contribute to the increased intratumoural T cell infiltration and increased response to immune checkpoint inhibitors observed in this subtype of melanoma[202].

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Box 2 |

Tissue-resident immune cells

Tissue-resident macrophages[80], γδ T cells[203], memory T (T_{RM}) cells[204] and innate lymphocytes[205] including natural killer (NK) cells[206,207] and mast cells[207] can persist in tissues for prolonged periods of time and contribute to shaping the tissue immune tone. The role of tissue-resident $CD8^+$ T_{RM} cells has been particularly studied in the skin[208–210] and their accumulation is associated with enhanced antitumour immunity in various human cancer types[211–213].

In response to threat from pathogens and injuries and likely also within tumour lesions, tissue-resident immune cells are activated locally and release inflammatory molecules. In the tumour context, these secreted inflammatory molecules may affect early tumour outcome and promote the recruitment of effector immune cells, thus contributing to shaping the microenvironment of tumour lesions.

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Uveal melanoma

The most common primary cancer of the eye in adults, arising from melanocytes located in the uvea (which comprises the choroid, the ciliary body and the iris).

Peripheral node addressin

(PNAd). A sulfated protein at the surface of high endothelial venules in secondary and tertiary lymphoid tissues that is crucial for the homing of naive and central memory T cells.

Lymphoid neogenesis

The de novo formation of ectopic lymphoid structures within peripheral tissues during chronic inflammation.

Lymphotoxin

A cytokine expressed by lymphoid tissue inducer cells and lymphocytes. in lymphoid neogenesis, lymphotoxin-α(LTα) forms a complex with LTβ to bind to the LTβ receptor on stromal cells, leading to nuclear factor-κB (NF-κB) signalling, which promotes the production of chemokines necessary for T cell and B cell recruitment.

Parabiosis

The surgical union of two organisms leading to the sharing of blood circulation, enabling the assessment of the recruitment and contribution of blood circulating cells to a cellular compartment in a tissue or lesion.

β-blockers

Drugs blocking β-adrenergic signalling on nerve cells, causing blood vessels to relax and dilate; these are commonly used to treat high blood pressure and other cardiac conditions.

Tumour agnostic

Compatible with any tumour type.

Multivalent vaccines

Vaccines designed to immunize against two or more antigens.

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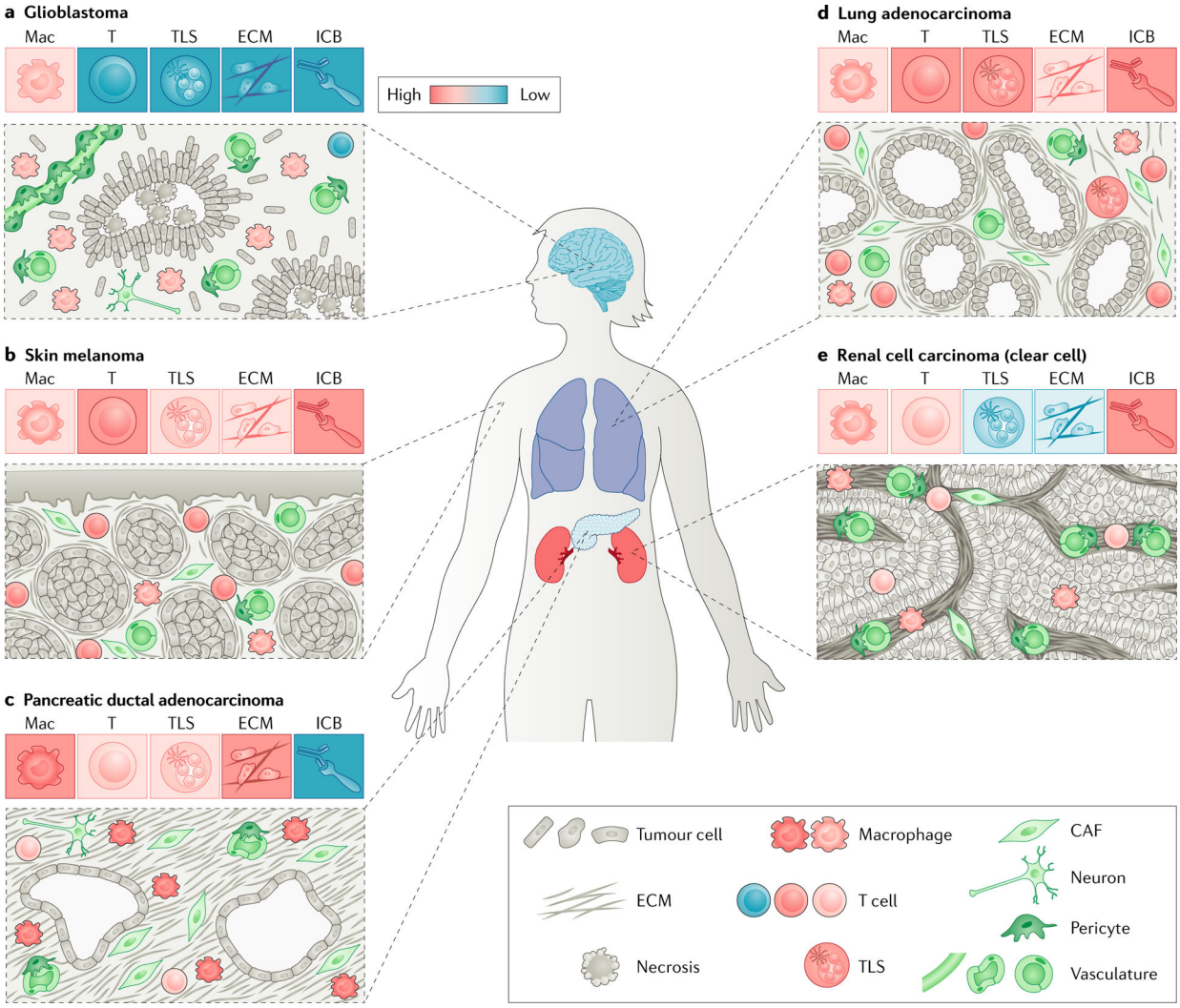

Fig. 1 |. The cellular and architectural heterogeneity of the tumour microenvironment at distinct cancer sites.

Schematics of representative histological patterns of glioblastoma (part **a**), skin melanoma (part **b**), pancreatic ductal adenocarcinoma (part **c**), lung adenocarcinoma (part **d**) and clear-cell renal cell carcinoma (part **e**) are shown. Tumour lesions at distinct tissue sites display different tumour mass organization, stroma to tumour ratios and levels of fibrotic reaction. In addition to neoplastic cells, each tumour microenvironment contains cells derived from both circulating cells and local cells such as fibroblasts, pericytes and endothelial cells that may differentially impact antitumour immune responses across cancer sites. For each tumour type, a colour-coded heatmap (red: high; blue: low) shows the level of dominance of macrophage or lymphocyte infiltrate, presence of tertiary lymphoid structures (TLSs), matrix deposition and response to immune checkpoint blockade (ICB). CAF, cancer-associated fibroblast; ECM, extracellular matrix;Mac, macrophage; T, T cell.

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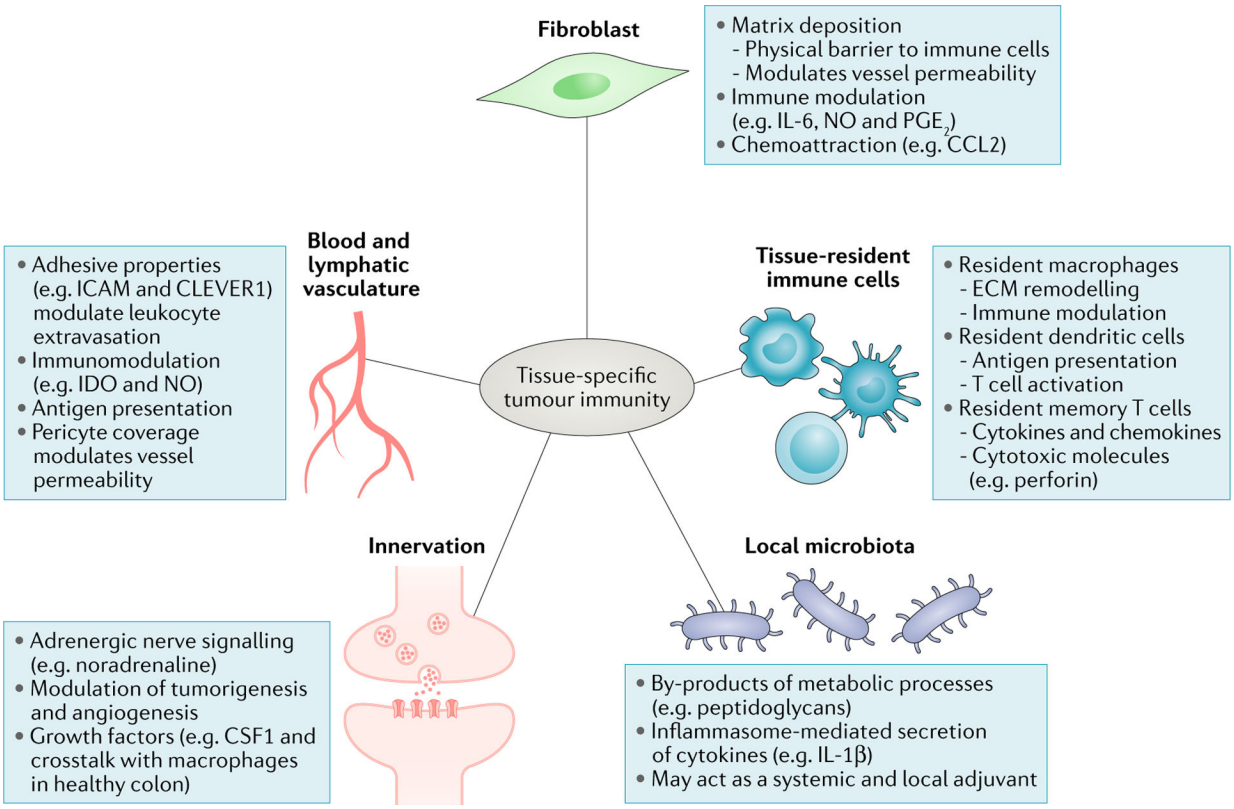

Fig. 2 |. Cellular contributors to tissue-specific antitumour responses.

Blood vessels across anatomical sites can differentially control tumour immune infiltrate through distinct levels of expression of adhesion molecules, cohesiveness and pericyte coverage, regulating immune cell extravasation. While the differential impact of lymphatic vessels on the immune infiltrate across cancer types is poorly understood, it is now well established that lymphatics help shape immune responses by promoting tolerance to self-antigens, archiving antigen for later presentation and dampening effector immune responses[214–217]. Cancer-associated fibroblasts modulate antitumour immune responses by secreting chemokines, cytokines, growth factors and reactive oxygen species, as well as producing and remodelling the stromal matrix that serves both a guiding and a barrier function for immune cells. Emerging evidence shows that commensal bacteria can set the tone of antitumour immune responses both systemically and locally, for example, by stimulating immune cells to secrete inflammatory cytokines. Tissue-resident cells, which include macrophages, dendritic cells and memory T cells, contribute to shaping antitumour immune responses by acting directly on tumour cells or modulating infiltrating immune cells. Nerve cells, whose presence has been reported in a limited number of cancer types, influence tumour cell survival, angiogenesis and the function of immune cells such as macrophages by releasing neurotransmitters and growth factors. CCL2, CC-chemokine 1igand2; CLEVER1, common lymphatic endothelial and vascular endothelial receptor 1; CSF1, colony-stimulating factor 1; ECM, extracellular matrix; ICAM, intercellular cell adhesion molecule; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; NO, nitric oxide; PG E2, prostaglandin E2.

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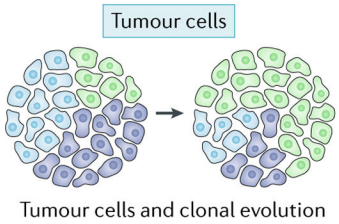

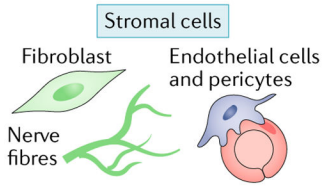

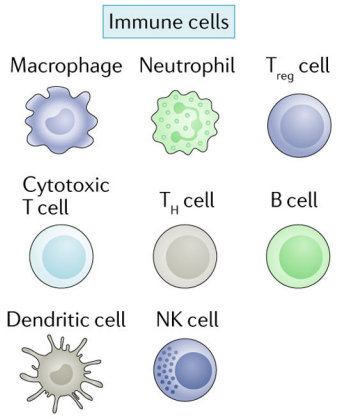

Tumour component	Mechanism and outcome	Tissue specificity	Proposed therapeutics
Tumour cells	Growth factors	Universal	Chemotherapy
	Hormone dependence	Unique	Hormone blockers
	Oncogene dependence	Unique	Kinase inhibitors
	HLA machinery loss	Universal	IFNγ
	IFNγ responsiveness	Universal	NA
	Chemokines	Universal	Blocking monoclonals
	Antigens	Unique	Vaccines
Stromal cells	Physical access barrier	Unique	Radiation therapy or anti-TGFβ
	Immunomodulation	Unique	NA
	Neurotransmitters	Unique	β-Blockers
Immune cells	Suppressive cytokines	Universal	Blocking monoclonals
	Metabolic depletion	Universal	Small molecules
	High myeloid density	Unique	Blocking monoclonals
	High granulocyte density	Unique	Blocking monoclonals
	Exhaustion	Universal	Checkpoint blockade
	Cytotoxicity	Universal	Checkpoint agonists
	Pro-inflammatory cytokines	Universal	Checkpoint agonists
	High lymphoid density	Unique	Checkpoints or cytokines
	TLS organization	Unique	Chemokines
	Cross-presentation	Universal	FLT3L or monoclonal agonists

Poor immune recognition and outcome | Favourable immune recognition and outcome | Already approved modalities

Fig. 3 |. Therapeutic implications of tumour cell-intrinsic and tumour cell-extrinsic factors dependent on tissue specificity.

Each tumour component has characteristics leading to poor or favourable immune recognition and outcome. Presence of the pin symbol in the column for proposed therapeutics indicates already approved modalities. FLT3L, FMS-like tyrosine kinase 3 ligand; HLA, human leukocyte antigen; IFNγ, interferon-γ; NK, natural killer; NA, not available; TGFβ, transforming growth factor-β; T_H cell, T helper cell; TLS, tertiary lymphoid structure; T_{reg} cell, regulatory T cell.