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Systemic immunity in cancer

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Abstract | Immunotherapy has revolutionized cancer treatment, but efficacy remains limited in most clinical settings. Cancer is a systemic disease that induces many functional and compositional changes to the immune system as a whole. Immunity is regulated by interactions of diverse cell lineages across tissues. Therefore, an improved understanding of tumour immunology must assess the systemic immune landscape beyond the tumour microenvironment (TME). Importantly, the peripheral immune system is required to drive effective natural and therapeutically induced antitumour immune responses. In fact, emerging evidence suggests that immunotherapy drives new immune responses rather than the reinvigoration of pre-existing immune responses. However, new immune responses in individuals burdened with tumours are compromised even beyond the TME. Herein, we aim to comprehensively outline the current knowledge of systemic immunity in cancer.

Peripheral immune cells
Immune cells in an individual bearing a tumour that are not in the tumour microenvironment.

Cancer is a systemic disease, and prolonged inflammation is a hallmark of cancer¹. Whether this inflammation initiates tumorigenesis or supports tumour growth is context dependent, but ultimately the global immune landscape beyond the tumour becomes significantly altered during tumour progression. Over the last decade, targeting the immune system with immunotherapy has revolutionized cancer therapy. Modulation of the existing patient immune system through immune checkpoint inhibitors (ICIs) such as anti-CTLA4, anti-PD1 and anti-PDL1 has led to durable remissions across a wide variety of different tumour types. Moreover, infusion of expanded autologous tumour-specific T cells or chimeric antigen receptor T cells has proven effective in patients with leukaemia. Despite these successes, immunotherapy remains ineffective for most patients with cancer^{2,3}. To date, most immunotherapies have largely been used in patients with advanced cancers, and therefore the response rate in less advanced disease remains to be fully determined. Further progress towards more broadly effective immunotherapeutic strategies requires a deeper understanding of the immunological relationships between tumours and their hosts across the body.

The tumour immunology field has focused heavily on local immune responses in the tumour microenvironment (TME), yet immunity is coordinated across tissues. For example, many myeloid cells are frequently replenished from haematopoietic precursors in the bone marrow⁴, and critical T cell priming events typically occur in lymphoid tissues⁵. The localized antitumour immune response cannot exist without continuous communication with the periphery. Furthermore, virtually every subset of immune cell has been implicated in cancer biology^{6,7}. Therefore, a thorough understanding

of immune responses to cancer must encompass all immune cell lineages across the peripheral immune system in addition to within the TME.

Recent clinical and preclinical studies are beginning to unravel the range of systemic immune perturbations that occur during tumour development as well as the crucial contribution of peripheral immune cells to an antitumour immune response. Here, we review recent advances that set the stage for a new holistic vantage point of tumour immunology to map and therapeutically harness the entirety of an immune response to cancer. We outline the extensive reorganization of peripheral immune cells that coincides with malignant tumour outgrowth as well as the systemic immunological consequences of conventional therapies (surgery, chemotherapy, radiation). We also examine the critical contribution of peripheral immune cells to driving and sustaining efficacious immunotherapy responses and the capacity of the tumour-burdened immune system to orchestrate a new immune response. Finally, we address the utility of peripheral immune biomarkers in aiding the diagnosis and prognosis of cancer and response to therapy.

Perturbations induced by tumour burden

Many human cancers and mouse models of cancer drive extensive disruption of haematopoiesis. This disruption manifests most prominently in an expansion of immature neutrophils and monocytes in the periphery of tumour-burdened hosts, which then also traffic to the TME and contribute to local immunosuppression. This phenomenon has been reviewed extensively elsewhere^{8–10}. In brief, haematopoietic stem and progenitor cells are mobilized into proliferation and differentiation towards the monocytic and granulocytic lineages, resulting in peripheral expansion and intratumoural

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Immune macroenvironment

The total immune system in a tumour-burdened host comprising blood and secondary lymphoid organs such as the bone marrow, spleen and lymph nodes.

Co-stimulatory and co-inhibitory receptors

Immunological receptors expressed on the surface of lymphocytes, antigen-presenting cells and tumour cells that stimulate or inhibit immune cell functions.

Lymphopenia

The reduced abundance of lymphocytes (T cells and/or B cells and/or natural killer cells) in the blood.

accumulation of immature immunosuppressive neutrophils (often referred to as polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs)), monocytes (often referred to as M-MDSCs) and macrophages^{11–15} (FIG. 1). Mouse models of breast cancer and rhabdomyosarcoma have demonstrated that the frequency of bone marrow haematopoietic stem cells, multipotent progenitors and granulocyte monocyte progenitors progressively increase with the tumour burden^{12,16–18}. Importantly, a pan-cancer study found elevated levels of haematopoietic stem cells, multipotent progenitors and granulocyte monocyte progenitors in the blood of patients with breast, cervical, liver, oesophageal, lung, ovarian and gastrointestinal cancers, suggesting that haematopoietic dysregulation is common in human cancer¹³. A comprehensive meta-analysis of more than 40,000 patients found that elevated neutrophil frequencies in the blood, as measured by the neutrophil to lymphocyte ratio, were associated with poor prognosis in patients with mesothelioma, pancreatic cancer, renal cell carcinoma, colorectal carcinoma, gastroesophageal cancer, non-small-cell lung cancer (NSCLC), cholangiocarcinoma and hepatocellular carcinoma¹⁹. Several factors have been implicated in driving this process including G-CSF^{12,20}, GM-CSF^{17,21,22}, IL-17 (REF.¹⁵), oxysterol²³, IL-8 (REF.²⁴), CCL2 (REF.¹⁴), TNF²⁵, tumour-derived exosomes²⁶ and IL-1 β (REF.²⁷).

The vast majority of research that highlights peripheral immune perturbations in the context of cancer has focused on this increase in immature and immunosuppressive myeloid populations; however, this expansion also often co-occurs with alterations to many other peripheral immune lineages (FIG. 1). Our group recently used mass cytometry to comprehensively profile the phenotype and frequency of all major immune lineages in the bone marrow, spleen, blood, draining lymph node (dLN) and tumour in eight distinct mouse tumour models²⁸. Although we observed a peripheral myeloid expansion in all tumour models as has been previously described, we also observed extensive peripheral immune reorganization across lineages and tissues. For example, three models of breast cancer (AT3, 4T1, MMTV-PyMT) spanning three different mouse strain backgrounds showed extensive splenic immune population remodelling characterized by phenotypic shifts as well as increased frequencies of neutrophils, eosinophils and monocytes along with reductions in dendritic cell, B cell and T cell populations (FIG. 1). Strikingly, surgical resection of the tumour or cytokine blockade treatments reversed many of the changes, suggesting plasticity in the peripheral reorganization of the immune macroenvironment in cancer. These data demonstrate that tumour development dramatically restructures the global immune landscape across immune cell lineages.

Beyond excessive production of monocytic and neutrophilic cells through aberrant haematopoiesis, perturbations in dendritic cells have been observed in the periphery of tumour-burdened hosts. This has important implications for the development of antitumour immune responses, as dendritic cells are critical orchestrators of CD8⁺ and CD4⁺ T cell priming, differentiation

and proliferation in many contexts, including cancer^{29,30}. The frequencies of dendritic cell subsets are decreased in peripheral blood of human ovarian³¹, prostate³¹, breast³², lung³³ and renal³⁴ cancers as well as head and neck squamous cell carcinoma³⁵ and melanoma³⁶ when compared with healthy control donors (FIG. 1). In patients with pancreatic or breast cancer and in mouse models of these cancer types, a decrease in the frequency of peripheral type 1 conventional dendritic cells (cDC1s) was driven through tumour-derived G-CSF, which caused a down-regulation of IRF8 in dendritic cell precursors, reducing the differentiation of mature dendritic cells¹⁸ (FIG. 1). Similarly, tumour-derived vascular endothelial growth factor (VEGF) has been shown to inhibit the maturation of dendritic cell precursors^{37,38}. An alternative mechanism for dendritic cell paucity in a mouse model of pancreatic cancer was shown to be mediated by serum IL-6 driving increased dendritic cell apoptosis³⁹ (FIG. 1). In patients with pancreatic cancer and mouse models of pancreatic cancer, peripheral dendritic cells differentiate into a semi-mature state characterized by moderate increases in co-stimulatory and co-inhibitory receptors^{39–41} (FIG. 1). Bulk transcriptomic analyses of these peripheral dendritic cells from mice bearing pancreatic tumours revealed that these semi-mature dendritic cells showed upregulation of genes involved in proteasomal degradation but did not show upregulation of T cell polarizing cytokines³⁹, suggesting that, similar to semi-mature dendritic cells in other contexts, they only partially possess the capacity to provide stimulation to T cells.

Substantially less is known about the organization of other major immune lineages in the tumour macroenvironment. Lymphopenia is common in patients with breast cancer, lymphoma and sarcoma⁴². Interestingly, circulating T cells in patients with breast⁴³, lung⁴⁴ and cervical⁴⁵ cancers have decreased diversity in the repertoire of T cell receptors (TCRs) (FIG. 1). As greater TCR diversity is associated with better tumour control in patients with melanoma⁴⁶, an improved understanding of TCR repertoire fluctuations driven by cancer is warranted. Furthermore, as a decreased TCR repertoire in humans is associated with age⁴⁷ as well as other prior immunological exposures such as chronic infection⁴⁸, these changes may also be a cause for malignant outgrowth. The causal relationship between TCR diversity and cancer has yet to be determined. Peripheral T cells are also functionally perturbed, as polyclonal memory CD4⁺ and CD8⁺ T cells from peripheral blood have decreased capacity to produce IL-2 and IFN γ in response to stimulation with PMA and ionomycin in human patients with breast cancer⁴⁹. Peripheral naive CD4⁺ T cells also exhibited decreased responses to IL-6 stimulation as measured by phosphorylation of STAT1 and STAT3 in patients with breast cancer⁵⁰ (FIG. 1).

The most studied perturbation of T cells in cancer is the expansion of suppressive CD4⁺ regulatory T (T_{reg}) cells in the periphery and their infiltration into the tumour⁵¹. Recent work has now shown that T_{reg} cells present in the blood of patients with cancer share phenotypic and TCR repertoires with intratumoural T cells, suggesting that a significant proportion of intratumoural suppressive T_{reg} cells are derived from naturally occurring

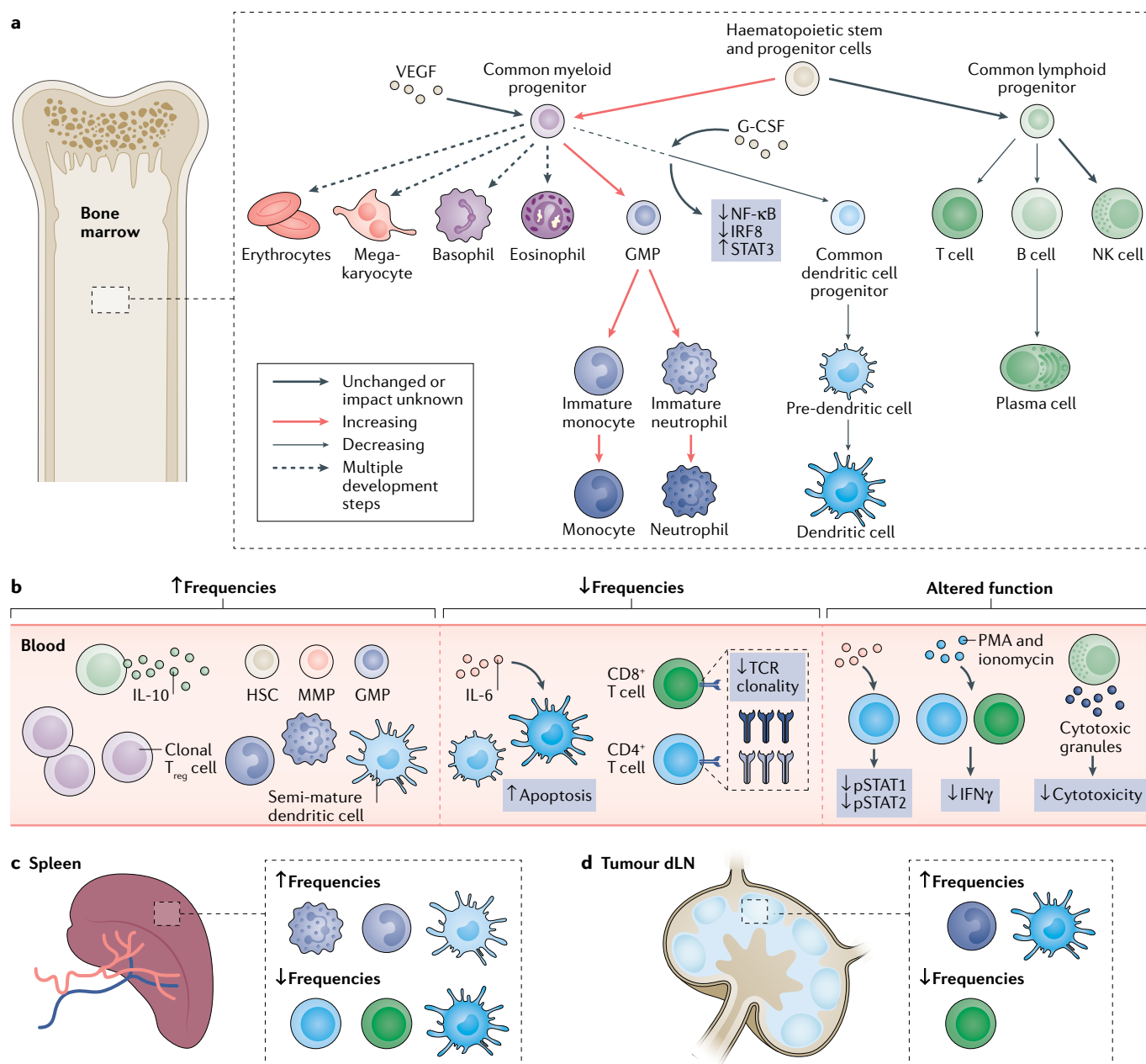


Fig. 1 | Systemic perturbations to immune organization by the tumour burden. The peripheral immune landscape is perturbed in many tumour types. The bone marrow, blood, spleen and draining lymph node (dLN) form an immunological network in constant communication during tumour development. **a** | Bone marrow haematopoiesis skews towards the production of neutrophils and monocytes through increased frequency of haematopoietic stem cells (HSCs) and granulocyte monocyte progenitors (GMPs). In some contexts, this skewing occurs at the expense of dendritic cell precursors which share progenitors, leading to a systemic paucity of dendritic cells that has been shown to be driven by G-CSF stimulating STAT3 signalling while repressing IRF8, as well as through vascular endothelial growth factor (VEGF) decreasing NF- κ B signalling. T cell, B cell and plasma cell populations in the bone marrow have also been shown to be decreased. **b** | During tumour development, bone marrow progenitor pools as well as suppressive immature monocytes and neutrophils are mobilized into circulation in the blood. Systemic increased frequencies of suppressive lymphocyte populations, CD4⁺ regulatory T (T_{reg}) cells and regulatory B cells are also commonly observed. T_{reg} cells specifically undergo clonal expansion in

the periphery before infiltrating the tumour. Dendritic cell as well as CD8⁺ and CD4⁺ T cell frequencies and T cell receptor (TCR) repertoire diversity are decreased in many tumour contexts. Functional deficits in response to stimuli have been identified in T cell populations. CD4⁺ T cells exhibit decreased signalling responses to IL-6 stimulation, and both CD4⁺ and CD8⁺ T cells produce less IL-2 and IFN γ in response to PMA and ionomycin stimulation. Natural killer (NK) cell cytotoxic potential is also decreased. **c** | Several alterations observed in the blood have been mirrored in the spleen in mouse models, including accumulation of immature neutrophils, monocytes and semi-mature dendritic cells. Decreased abundance of dendritic cells and T cell populations has also recently been described. **d** | The tumour dLN has the most direct line of communication with the tumour and is characterized by increased frequency of monocytes and dendritic cells with a decrease of CD8⁺ T cells. Collectively, these observations across many human and mouse tumour models demonstrate that the peripheral immune landscape is shifted towards a suppressive state marked by increases in anti-inflammatory cell types and decreases in key mediators of antitumour immunity. MMP, multipotent progenitor.

thymic T_{reg} cells rather than through tumour-induced differentiation of naive CD4⁺ T cells^{52,53} (FIG. 1).

Another suppressive lymphocyte population that plays a role in tumour progression is regulatory B cells, which are characterized by production of the anti-inflammatory cytokine IL-10 (REF.⁵⁴). Similar to T_{reg} cells, an expansion of IL-10-producing regulatory B cells has been documented in peripheral blood of patients with gastric cancer^{55,56} and patients with lung cancer⁵⁷, whereas frequencies of total B cells remained unchanged (FIG. 1). In the 4T1 mouse model of breast cancer, suppressive CD25⁺ regulatory B cells were expanded in the spleen, lymph nodes and blood⁵⁸.

Natural killer (NK) cells are yet another important component of antitumour immunity that can directly kill tumour cells as well as influence antitumorigenic behaviour of other immune cells⁵⁹. Peripheral NK cells from patients with breast cancer also have altered phenotypes, characterized by decreased expression of activating receptors, including Nkp30, NKG2D, DNAM-1 and CD16, and increased expression of the inhibitory receptor NKG2A, as well as impaired capacity to directly kill target cells and degranulate *in vitro*⁶⁰. In patients with gastrointestinal stromal tumours, peripheral NK cells showed decreased expression levels of the activating receptor Nkp30 and impaired degranulation upon Nkp30 cross-linking. Paradoxically, NK cells from patients with gastrointestinal stromal tumour produced more IFN γ upon either IL-2 stimulation or incubation with dendritic cells, the latter of which predicted improved response to imatinib mesylate treatment⁶¹. In NSCLC, transcript levels of activating receptors NCR1, NCR2 and NCR3 in peripheral NK cells are all decreased, reflecting impaired natural killer cell activation, and low NCR3 transcript expression and elevated serum levels of its ligand, B7-H6, were associated with poor survival⁶². In neuroblastoma, patients with metastatic disease exhibited lack of expression of the A and B isoforms of Nkp30 in peripheral NK cells when compared with patients with localized disease and healthy individuals⁶³. Conversely, another study in patients with NSCLC found that peripheral NK cells showed no phenotypic alterations by flow cytometry compared with cells from healthy individuals, but *ex vivo* incubation of these NK cells with tumour cells induced reduction of NK cell receptor expression and impaired degranulation compared with healthy donor-derived NK cells, suggesting that, in some contexts, NK cell perturbations are specific to the TME⁶⁴.

Altogether, these data strongly support the notion that systemic corruption of immune organization occurs across diverse tumour types (FIG. 1; TABLE 1). Further work is needed to fully characterize the distinct types of immune states in patients with cancer and the associations of these types of immune states with the tumour tissue of origin, stage of development and patient demographics in order to inform therapeutic development and future mechanistic studies of the causes of systemic disruptions. It is also critical to understand why systemic immune changes are quite dramatic in some contexts yet subtle in others.

Changes induced by conventional therapy

Conventional therapeutic strategies in cancer, including chemotherapy, radiation and surgery, perturb the global immune landscape. Understanding these systemic immune consequences is important for designing strategies that augment rather than impede antitumour immune responses, which can include optimal timing, dosing or combinations.

Chemotherapy and radiation therapy remodel circulating immune populations. Chemotherapy and radiation therapy are designed to target cancer cells by compromising cellular integrity during division; however, these agents can also induce remodelling of immunity that can either impede or augment overall treatment efficacy. Consequences of conventional cancer therapies on the immune system were well reviewed by Shaked, such as expansion of immunosuppressive myeloid cells via elevated pro-inflammatory cytokines, including IL-6, IL-8 and GM-CSF, and B cell release of systemic extracellular vesicles that impede antitumour cytotoxic immune functions⁶⁵. One counter-strategy is to pair these therapies with agents that block immunosuppressive phenotypes, such as inhibiting CSF1R or CCR2. Chemotherapeutic cytotoxicity also leads to general lymphodepletion, and although the numbers of CD8⁺ T cells in peripheral blood fully rebound to normal frequencies within a year, an abnormal bias of memory CD4⁺ T cells towards inflammatory effectors persists for years in patients with breast cancer⁶⁶. Selecting agents that mitigate immune abnormalities may be optimal for enabling the strongest antitumour immune response.

The impact of chemotherapy and radiotherapy on the immune system depends highly on context, making it challenging but imperative to understand how each cytotoxic therapy may compromise immune function across cancer settings. In NSCLC, standard prolonged low-dose radiotherapy, but not chemotherapy, led to myeloid cell expansion, reduced antigen-presenting cell function and impaired T cell responses⁶⁷. Similar immune impacts were observed after combination chemotherapy and radiotherapy in patients with cervical cancer⁶⁸. Neoadjuvant chemotherapy prior to surgical resection is a strategy often used in breast cancer, but patients show disparate immune effects depending on the cancer stage and therapeutic agent. In patients with non-metastatic breast cancer, doxorubicin and cyclophosphamide chemotherapy led to elevated numbers of circulating PMN-MDSCs but no changes in M-MDSCs when compared with pretreatment numbers⁶⁹. However, in patients with metastatic breast cancer treated with 5-fluorouracil (5-FU), epirubicin and cyclophosphamide (FEC) or docetaxel chemotherapies, M-MDSCs were dramatically reduced in six out of ten patients when compared with pretreatment levels⁷⁰. Specifically, in patients with breast cancer with tumours expressing HER2 (HER2⁺), a recent study suggests that higher circulating IL-10 and classical monocytes associate with reduced pathological complete responses after chemotherapy⁷¹. Future studies with larger numbers of patients and more complete measurements of the immune system are needed to

Table 1 | Peripheral immune perturbations in cancer

Immune cell type	Change	Tumour type and species ^a	Refs
Haematopoietic stem cells	Increased frequency	Human: breast cancer, cervical cancer, liver cancer, oesophageal cancer, lung cancer, ovarian cancer, gastrointestinal cancer Mouse: breast cancer ^M , rhabdomyosarcoma ^M	12,13,16,17
Multipotent progenitor cells	Increased frequency	Human: breast cancer, cervical cancer, liver cancer, oesophageal cancer, lung cancer, ovarian cancer, gastrointestinal cancer Mouse: breast cancer ^M , rhabdomyosarcoma ^M	12,13,16,17
Granulocyte monocyte progenitors	Increased frequency	Human: breast cancer, cervical cancer, liver cancer, oesophageal cancer, lung cancer, ovarian cancer, gastrointestinal cancer Mouse: breast cancer ^M , rhabdomyosarcoma ^M	12,13,16,17
Dendritic cell precursors	Decreased frequency	Human: breast cancer ^{NM} , pancreatic cancer Mouse: breast cancer, pancreatic cancer	18
Immature neutrophils/PMN-MDSCs	Increased frequency	Human: breast cancer, pancreatic cancer, lung cancer ^{M/NM} , bladder cancer ^{M/NM} , head and neck cancer ^{M/NM} , glioblastoma, melanoma Mouse: breast cancer ^M , melanoma ^M , pancreatic cancer ^M , colon cancer ^{NM} , glioblastoma	11,12,18, 28,160–163
Immature monocytes/M-MDSCs	Increased frequency	Human: renal cell carcinoma, prostate cancer, prostate cancer, melanoma ^{M/NM} , hepatocellular carcinoma Mouse: breast cancer ^{M/NM} , melanoma ^M , pancreatic cancer ^M , colon cancer ^{NM} , glioblastoma	14,22,28, 164–167
Dendritic cells	Decreased frequency	Human: ovarian cancer, prostate cancer, breast cancer, head and neck squamous cell carcinoma, melanoma ^{M/NM} , lung cancer ^{NM} , renal cancer Mouse: pancreatic cancer ^M , breast cancer ^{M/NM} , glioblastoma	11,18,28, 32–34,36,168
T cells	Decreased TCR repertoire	Human: breast cancer ^M , lung cancer ^{M/NM} , cervical cancer	43–45
T _{reg} cells	Expansion	Human: lung cancer ^{M/NM} , prostate cancer ^{NM} , gastric cancer ^{M/NM} , colorectal cancer, oesophageal cancer, hepatocellular carcinoma, pancreatic cancer, breast cancer ^{NM} Mouse: melanoma, pancreatic cancer	28,51, 169–174
T _{reg} cells	Clonal expansion	Human: melanoma ^M , gastrointestinal cancer, ovarian cancer, breast cancer	52,53
Regulatory B cells	Increased frequency	Human: gastric cancer ^{M/NM} , lung cancer Mouse: breast cancer ^M	55–57
CD4 ⁺ and CD8 ⁺ T cells	Decreased IL-2 and IFN γ production after PMA and ionomycin stimulation	Human: breast cancer ^{M/NM}	49
CD4 ⁺ T cells	Decreased pSTAT1 and pSTAT3 signalling after IL-6 stimulation	Human: breast cancer	50
Natural killer cells	Decreased activating receptors, increased inhibitory receptors, decreased cytotoxic potential	Human: breast cancer ^{M/NM} , lung cancer ^{NM} , gastrointestinal cancer ^{M/NM} , neuroblastoma ^M	60–64

M-MDSC mononuclear myeloid-derived suppressor cell; PMN-MDSC, polymorphonuclear myeloid-derived suppressor cell; TCR, T cell receptor; T_{reg} cell, regulatory T cell. ^aSuperscript ^M indicates that this observation was specifically made in metastatic disease, whereas ^{NM} indicates that the observation was specifically made in non-metastatic disease.

parse how the disease type and stage affect the immune consequences of cytotoxic therapies.

When demonstrably effective, chemotherapy can augment systemic antitumour immunity in conjunction with disrupting cancer cell division. Recent work showed that effective responses to pre-surgical

neoadjuvant chemotherapy in triple-negative breast cancer (TNBC) induces the recruitment of new T cell clones to the TME rather than expanding those already present⁷². Importantly, different subtypes of breast cancer showed differential immune responses to this therapeutic strategy, reflected in the functionality of peripheral

CD8⁺ T cells. Patients with oestrogen receptor-positive (ER⁺) breast tumours had a drop or stasis in the poly-functionality of circulating PD1⁺CD8⁺ T cells, measured by cytokine production after TCR stimulation. Patients with ER⁺HER2⁺ breast tumours showed a complete loss of functionality in this subset. Conversely, patients with TNBC showed elevated PD1⁺CD8⁺ T cells with high functionality, producing effector cytokines including IFN γ and TNF, and the cytolytic molecule granzyme B, and with evidence of clonal expansion. Ultimately, tumour-infiltrating T cells were only prognostic for overall survival in TNBC. Moreover, a cytolytic but exhausted CD8⁺ T cell signature in the blood of patients with TNBC following chemotherapy was associated with ongoing disease and was predictive of recurrence or metastasis post surgery.

With the advent of immunotherapy, therapeutic strategies are shifting towards utilizing cytotoxic therapeutic agents that can augment antitumour immunity, such as by disrupting the tumour stroma or by releasing tumour antigens for de novo activation of the adaptive immune system^{73–75}.

Tumour resection can impact immunological control of cancer. Recent studies have provided a deeper understanding of the impact of surgical tumour resection on the systemic immune state and immunological control of metastases. Metastatic outgrowth following surgical tumour resection has been documented in several cancer types, where a wide range of pro-tumorigenic processes, including shedding of tumour cells into circulation and stimulation of angiogenesis, lead to new and accelerated metastatic growth despite resection of the primary tumour⁷⁶. For the purposes of this Review, we focus on alterations to antitumour immunity following surgery (other aspects driving postoperative metastasis are reviewed elsewhere⁷⁷). Several recent studies implicate myeloid immune cell remodelling induced by systemic wound healing programmes. Resection, or wounding independent of primary tumour removal, triggers healing programmes that elevate circulating IL-6, G-CSF and CCL2, and ultimately drive myeloid subsets towards immunosuppressive states⁷⁸. Although resection substantially reduces the number of systemic MDSCs in the spleen, blood and lung in the 4T1 breast cancer model, functional immunosuppressive PMN-MDSCs can persist in these peripheral tissues for 2 weeks⁷⁹. Persistent immunosuppressive myeloid cells were shown to support pro-tumorigenic niches in the lungs in both breast cancer and osteosarcoma models^{79,80}. Mechanistically, one study showed that neutrophil extracellular traps increased in the liver following surgical intervention and ensnared tumour cells to promote metastasis⁷⁷. Modulation of myeloid subsets in the adjuvant setting can prevent post-surgical metastases, including the use of gemcitabine to deplete PMN-MDSCs⁷⁹ or gefitinib to alter inflammatory macrophage states by blocking signalling through receptor-interacting protein kinase 2 (RIPK2)⁸⁰. Surgical procedures in a mouse tumour vaccination model showed that tumour-specific T cell responses were dramatically weakened for 7–10 days following surgery⁸¹. Similarly, a mouse model of surgical

stress, in which mice underwent an abdominal laparotomy and left nephrectomy, showed that surgery led to decreased systemic NK cell frequencies and tumour killing potential, which culminated in impaired control of lung metastasis⁸². Patients with colorectal cancer also exhibited decreased IFN γ secretion from peripheral NK cells when compared with healthy individuals, and this was further decreased for up to 2 months following surgery⁸³. Collectively, these data suggest multiple mechanisms by which surgical resection induces global immunological perturbations that can promote metastasis.

However, our group recently demonstrated that the primary tumour can be the main driver of systemic immune remodelling: successful primary tumour resection in mouse models of breast and colon cancer was sufficient to largely restore normal systemic immune organization to immune cell frequencies comparable with healthy control mice across the spleen, lymph node, blood and bone marrow given that there was sufficient time to recover from postoperative complications²⁸. In the highly metastatic 4T1 mouse model, lung metastatic outgrowth was observed in some animals following surgery and yet only minor systemic immune changes were maintained. Furthermore, we and others found that surgical tumour resection in mice ultimately restored functional orthogonal responses to infection, vaccination or allogeneic tumour challenges^{28,84}. Therefore, it is likely that surgery results in both detrimental and beneficial effects on the systemic immune system. Immunosuppressive mechanisms coinciding with wound healing early after surgery potentially provide a window of opportunity for disseminated cancer cells to grow out. However, the reduced primary tumour burden can ultimately restore systemic immune capacity for strong adaptive responses. It will be important to discover how the cancer type and, particularly, disease stage influence immune remodelling following surgery and the resulting potential for metastasis.

Appropriate pairing of conventional therapies with immune modulation can be a powerful tool to combat cancer, and taking the systemic immune context into account is likely to result in improved outcomes. It is particularly important to consider the immunologically vulnerable period of time following surgery and further investigate the mechanisms driving these states as well as potential therapeutic interventions to restore immune function and prevent tumour recurrence and metastasis.

Systemic responses in immunotherapy

Cancer immunotherapy has radically expanded our toolkit against cancer, with current US Food and Drug Administration (FDA) approval of 7 ICIs across 19 different cancer types, in addition to chimeric antigen receptor T cells, bispecific T cell engager (BiTE) therapies and vaccines. The prevailing view of cancer immunotherapy efficacy has centred around the notion of reinvigorating cytotoxic effectors within the TME, but appreciation is growing in the field for the fundamentally systemic nature of effective antitumour immunity. Recent studies demonstrate that ICIs, including blockade of the PD1 and PDL1 axis, rely on systemic immune

Neutrophil extracellular traps

Extracellular web-like structures comprising DNA and cytosolic and granule proteins created by neutrophils to trap and neutralize invading pathogens.

Orthogonal responses

Immunological challenges that do not share any antigens with the tumour.

Box 1 | Microbiome modulation of systemic immunity in cancer

The activity and composition of the microbiome influences the organization of the human immune system¹⁷⁵. Antibiotic treatment that disrupts the gut microbiome leads to resistance to immune checkpoint inhibitors (ICIs) in mouse models of cancer and in patients with cancer¹⁷⁶. Multiple studies have found that faecal microbiota transplantation (FMT) from patients into mouse models can recapitulate functional outcomes on tumour control and response to ICIs such that FMT from ICI responders drives improved antitumour immune responses compared with FMT from ICI non-responders^{176–180}. In fact, FMT from ICI non-responders compared with responders drove divergent peripheral immune responses, such as higher frequencies of regulatory CD4 T cells and T helper 17 cells in the spleen of non-responder FMT recipients, suggesting systemic consequences of microbiome composition in patients with cancer¹⁷⁷. Although much mechanistic work is still needed to link microbiome to immune composition and function, *Bifidobacterium pseudolongum* and *Akkermansia muciniphila* have been shown to produce inosine that activates antitumour T cells via the adenoside A_{2A} receptor¹⁷⁹. One study identified an 11-strain mixture of commensal bacteria that enhances antitumour immune responses through CD103⁺ dendritic cell-orchestrated CD8⁺ T cell responses. Systemically, the same 11-strain mixture also drove enhanced intestinal bacterial clearance following oral *Listeria monocytogenes* infection as well as improved spleen and liver bacterial clearance after intraperitoneal *L. monocytogenes* infection¹⁸⁰. These data suggest that microbiome-based improvements of antitumour immunity also shape systemic immunity. This topic has been reviewed in greater depth elsewhere¹⁸¹.

mechanisms to achieve effective antitumour responses. Furthermore, the microbiome is emerging as a potent modulator of the immune system, and the relevant impact on antitumour immune responses is discussed in BOX 1.

Intact peripheral immunity is critical for immunotherapeutic efficacy. Intact peripheral immune function, communication and trafficking are required for ICI efficacy. Disruption of peripheral immune integrity by systemic chemotherapy can impede therapeutic benefit by PD1 blockade, causing systemic lymphodepletion and abrogating long-term immune memory⁸⁵. By contrast, local chemotherapy spares peripheral immunity, collaborating with PD1 blockade to induce dendritic cell infiltration into the tumour and clonal expansion of antigen-specific effector T cells⁸⁵. A specialized subset of CD103⁺ dendritic cells transport tumour antigen to the peripheral immune system by CCR7-dependent migration from the tumour to the dLN, where the priming of tumour-specific CD8⁺ and CD4⁺ T cells occurs^{86–89} (FIG. 2). cDC2s are also capable of trafficking tumour antigen to the dLN and priming tumour-specific CD8⁺ and CD4⁺ T cells; however, this process is often restrained by intratumoural T_{reg} cells^{30,90}. Recent evidence suggested that dendritic cells migrating from the tumour to the dLN can transfer antigen to lymph node-resident dendritic cells that can then also prime tumour-specific T cells⁹⁰ (FIG. 2). Newly primed tumour-specific T cells then traffic from the lymph node to the tumour, and this cycle is an essential process in natural and therapeutically induced antitumour immunity⁹¹. As further evidence of the systemic nature of antitumour immunity, blockade of lymphocyte egress from lymphoid organs or surgical resection of tumour dLNs abrogates immunotherapeutic efficacy^{92,93}. The eradication of systemic disease also heavily relies on global immune responses. Strong adaptive immune responses confer peripheral memory, where the transfer of T cells from secondary lymphoid organs (including the spleen, lymph node and

blood) after productive antitumour responses is sufficient to protect naive animals⁹². This same study showed that systemic PDL1 blockade can break tolerance to disseminated tumours when paired with local therapeutic delivery at one site.

It has become clear that inhibiting the PD1–PDL1 axis has impacts beyond blocking local immunosuppressive cues in the tumour, and recent work has clarified key peripheral immune cells driving responses in these settings. First, therapeutic benefit of immune checkpoint inhibition is only observed in models with intact host PD1 and PDL1 expression and is less dependent on cancer cell expression of PDL1 (REFS^{94–96}). Aside from tumour cells, the majority of cells that express PDL1 are antigen-presenting cells, including macrophages and, at even higher levels, cDCs⁹⁷. In patients with melanoma or ovarian cancer, expression levels of PDL1 on intratumoural macrophages and cDCs correlate with clinical complete responses to anti-PDL1 and anti-CTLA4 therapy⁹⁵. Moreover, several groups have recently demonstrated that dendritic cells are a critical mediator of PDL1 blockade efficacy^{97–100}. Targeted depletion of PDL1 in cDCs, but not macrophages, substantially reduced CD8⁺ T cell responses and tumour shrinkage in response to PDL1 blockade in the subcutaneous MC38 mouse cancer model⁹⁷. The critical location for this interaction appears to be the tumour dLNs, as tumour-specific PD1⁺ T cells in the dLN showed high co-localization with PDL1-expressing cDCs⁹⁸. Selective targeting of PDL1 engagement in the dLNs was sufficient to induce effective antitumour responses across two syngeneic models, albeit to a lesser extent than systemic PDL1 blockade⁹⁸. Further supporting the significance of PDL1 activity specifically on cDCs, interactions between PDL1⁺ cDCs and PD1⁺ T cells in dLNs were indicative of the disease dissemination status in patients with melanoma. Frequent PD1 and PDL1 interactions in dLNs were observed in patients with metastatic melanoma and were predictive of early disseminated disease recurrence in patients with non-metastatic melanoma⁹⁸. Augmenting effector and memory T cell development in the dLN via mitochondrial activation further improved PD1 blockade efficacy in tumour-bearing mice¹⁰¹, again highlighting that systemically engaged immunity is clearly optimal for tumour eradication.

Effective immunotherapies drive de novo immune responses. Productive antitumour responses ultimately necessitate functional effector lymphocytes within the TME to mediate cancer cell killing. However, recent studies revealed that intratumoural T cells acquire terminally exhausted states over time, rendering them incapable of key effector functions (FIG. 2). Analysis of epigenetic landscapes of CD8⁺ T cells in mice and patients with cancer showed that intratumoural T cells underwent extensive chromatin remodelling, which locked cells in dysfunctional states and reduced the ability of these cells to produce TNF and IFN γ ¹⁰². This process was biphasic in preclinical models, where early T cell remodelling was reversible upon removal from the tumour context, but a second wave of epigenetic remodelling led to irrecoverable T cell dysfunction marked by

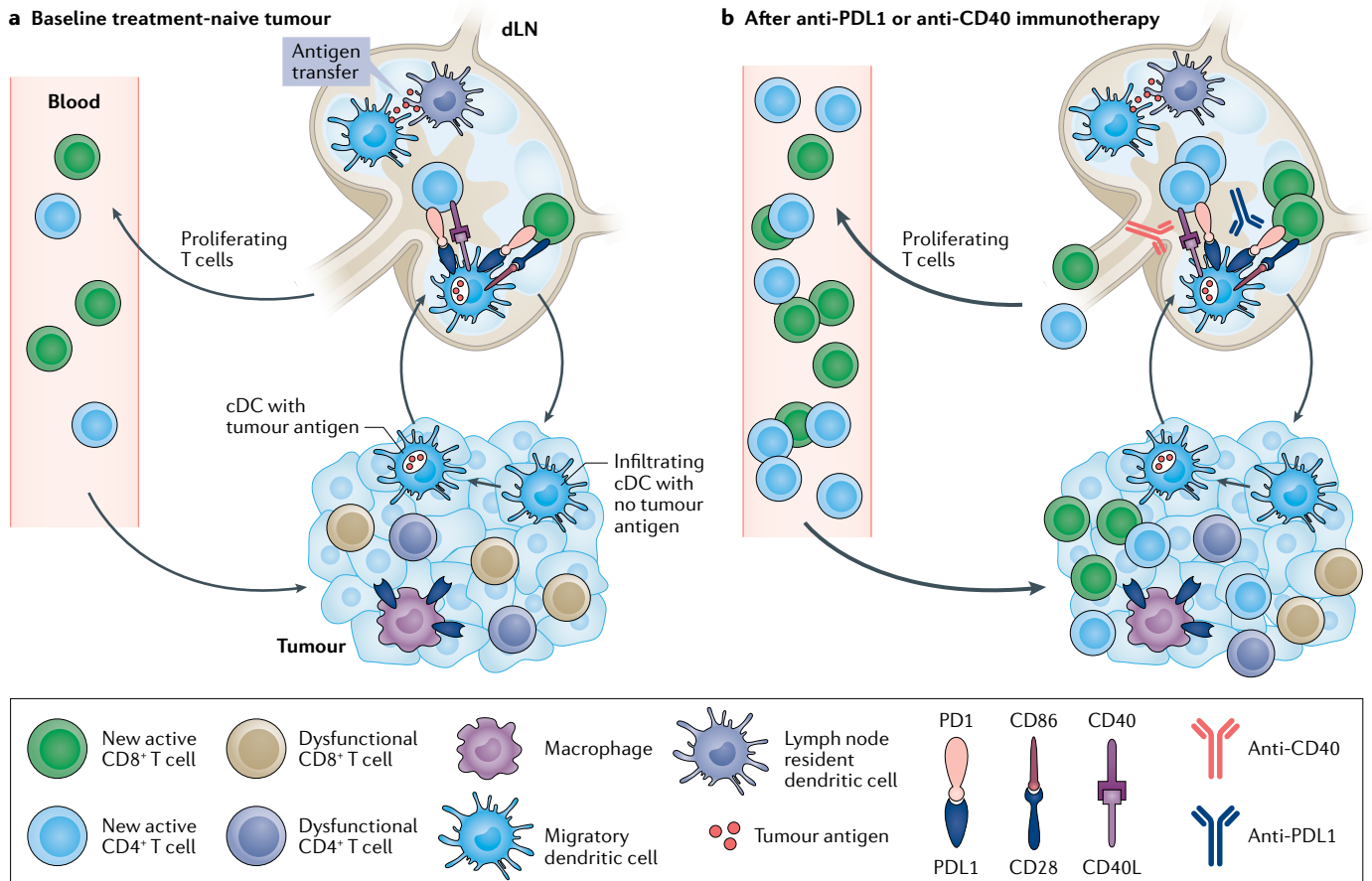


Fig. 2 | Systemic immune responses in cancer immunotherapy. Effective responses to immunotherapy drive de novo peripheral immune responses. Schematic illustrating how functional antitumour responses are reliant on immune dynamics outside the tumour microenvironment (TME). **a** | At baseline, conventional dendritic cells (cDCs) in the TME take up tumour antigen and travel to the draining lymph node (dLN), where they can then transfer antigen to resident cDCs through the formation of direct synapses. T cells in the TME reach states of terminal exhaustion due to chronic stimulation, the harsh environment and immunosuppressive cues. Dysfunctional intratumoural T cells accumulate structurally damaged mitochondria, and upregulate CD103 and CD38 coinciding with irreversible epigenetic remodelling. Thus, effective antitumour responses driven by

therapy must rely on another source of functional effector T cells. **b** | Immunotherapeutic intervention through PD1 and PDL1 checkpoint blockade increases the interaction between cDCs and naïve T cells in the dLN, and, alongside CD28 co-stimulation, facilitates the priming and rapid expansion of new T cell clones with new antigen specificities. Checkpoint blockade also leads to the proliferation of existing T cell clones in circulation. These expanding peripheral T cells ultimately infiltrate the TME, and express markers indicative of antigen-specific activation and demonstrate functional cytotoxicity. Productive de novo immune responses can also be achieved through CD40 agonism, which can drive cDC activation in settings resistant to checkpoint blockade and initiate these new T cell responses to replace exhausted intratumoural clones.

CD101 and CD38 co-expression¹⁰². Additional studies have identified the transcription factor TOX as a critical regulator in transcriptional and epigenetic reprogramming in response to chronic T cell stimulation, leading to T cell exhaustion^{103,104}. This intratumoural T cell dysfunction is driven by microenvironmental stressors in the TME, chronic TCR stimulation and checkpoint protein signalling^{103–106}. A study recently showed that metabolic challenges within the tumour can cause T cells to accumulate structurally damaged mitochondria with high levels of reactive oxygen species and overall compromised membrane potential¹⁰⁵. Importantly, mitochondrial dysregulation was sufficient to induce epigenetic reprogramming towards terminal dysfunction and was not observed in peripheral T cells from the spleen or dLN. Clinical responses to ICIs in patients with melanoma were associated with the presence of a stem-like CD8⁺ T cell state with reduced expression of co-inhibitory molecules and elevated memory, activation

and cell survival transcriptional and epigenetic programmes compared with exhausted CD8⁺ T cells¹⁰⁷. These productive intratumoural CD8⁺ T cells can be identified by expression of the transcription factor TCF1 (which is involved in WNT signalling in stem cell-like memory programmes) and notable lack of expression of CD39 and TIM3 (REF.¹⁰⁷). Thus, immunotherapy efficacy relies on the quality of effector CD8⁺ T cells within the TME, but persistence in this toxic microenvironment rapidly drives dysfunctional differentiation of T cells that lose their ability to efficiently contribute to tumour clearance.

To overcome local immune dysfunction, effective immunotherapies drive de novo peripheral immune responses culminating in new effector T cell infiltration (FIG. 2). Several reports have now shown that PD1 and PDL1 blockade drive novel T cell clones into the TME that were not present locally prior to therapy^{108–110}. In a recent study in patients with basal cell carcinoma

T cell exhaustion

A terminal state of T cell differentiation driven by chronic T cell receptor (TCR) stimulation and characterized by expression of inhibitory receptors and hypofunctionality, including a reduced capacity to secrete cytokines.

before and after PD1 blockade, 68% of all intratumoural CD8⁺ T cell clones after PD1 blockade were novel, 84% of which displayed exhaustion markers indicative of antigen-specific activation, and these cells represented novel TCR specificity groups, suggesting priming against new antigen targets¹⁰⁸. Further, 35.5% of these novel clones were also detected in the blood, with 11.8% detected in circulation pretreatment. Correlation between T cell clones in the blood and tumour was also demonstrated in patients with metastatic melanoma, renal cancer, lung cancer and colon cancer^{109,110}. Anti-CTLA4 has also been shown to dramatically increase peripheral T cell reactivities in patients with melanoma, suggesting new T cell priming as a mechanism of action¹¹¹.

Mechanistically, cell-intrinsic CD28 signalling in CD8⁺ T cells is critical in PD1 blockade efficacy^{112,113}, providing necessary co-stimulation for naive T cell priming. In line with this finding, a higher baseline proportion of CD28⁺CD57⁺KLRG1⁺ senescent CD8⁺ T cells in the blood of patients with NSCLC was associated with resistance to ICIs¹¹⁴. Impressively, in patients with classical Hodgkin lymphoma, the peripheral T cell clonal diversity at baseline was associated with PD1 blockade efficacy, illustrating how individual systemic immune contexts dictate the impact of immunotherapeutic intervention¹¹⁵. This signature was complemented by greater expansion of singleton clones in the blood of patients with complete response, likely representing peripheral T cells that had not encountered antigen pretreatment. The antitumour immune response in this cancer context was more reliant on CD4⁺ T cells, with expanded CD4⁺ TCR diversity, and concordant associations with circulating B cell abundance and a novel innate effector population capable of antibody-dependent cellular cytotoxicity. Together, these results support the notion that not only is peripheral immunity involved in renewed antitumour responses but also de novo priming of additional naive T cells with new antigen specificities contributes to effective immunotherapy (FIG. 2).

Intentional strategies for driving de novo immune responses are gaining traction in clinical trials, including stimulation of dendritic cell activity through various strategies such as agonistic CD40 antibodies^{116,117}. Several studies have demonstrated that immune checkpoint blockade relies on derestricting cDCs to allow for effective T cell priming^{97–100}, but this strategy fails in cases where there is a poor or absent pre-existing activation of antigen-presenting cells. Patients with pancreatic cancer are resistant to ICIs, but preclinical models demonstrate that combination with CD40 agonism can produce complete pancreatic tumour regression and extend survival independent of TLR, STING or IFNAR signalling¹¹⁸. Efficacy in this particular *Kras*^{LSL-G12D/+}, *Trp53*^{LSL-R172H/+}, *Pdx1*-Cre model was dependent on host BATF3⁺ cDC1s and CD40, as well as effector CD4⁺ and CD8⁺ T cells, which massively expanded in the blood and dLNs, indicative of peripheral immune activation¹¹⁸. An early clinical trial in patients with metastatic pancreatic cancer holds promise, where combination of CD40 agonism with PD1 blockade and gemcitabine and nab-paclitaxel chemotherapy

shows a greater than 50% objective response rate and the induction of proliferating CD4⁺ and CD8⁺ T cells in the blood¹¹⁹. CD40 activation is just one strategy demonstrating that converting immunologically ‘cold’ tumour contexts into ‘hot’ immune involvement requires de novo immune responses rather than reinvigoration.

Secondary immune challenges in cancer

The prior experience and state of the immune system dramatically shapes future responses to new challenges. Altered basal cytokine levels, cellular composition and cellular activation states are known to impact the nature and magnitude of secondary responses in models of chronic infection and co-infection^{120–122}. As the systemic immune state is significantly reorganized in individuals bearing tumours, this may have functional consequences on the orchestration of new immune responses. Identifying systemic functional deficits to immunological challenges, such as vaccines or infections, in patients with cancer remains challenging due to the effects of common cancer therapies. Patients with cancer are capable of developing detectable antibodies in response to influenza vaccination that are comparable with healthy individuals¹²³. However, during the ongoing 2020 SARS-CoV-2 pandemic, patients with cancer who are infected with SARS-CoV-2 are more likely to develop severe symptoms and exhibit higher mortality rates^{124,125}. Notably, even infected patients not receiving cancer treatment were at increased risk of mortality and severe illness¹²⁵. This observation suggests that the substantial phenotypic and compositional changes to the systemic immune system across many cell types could lead to altered immune responses to a secondary challenge outside the TME. As an intact functional peripheral immune system is critical for the development of new antitumour immune responses, as described in the previous section, it is imperative to understand how immunological decisions are made within the context of a tumour-burdened state.

Recent work has begun to mechanistically dissect why the tumour-burdened immune state results in weakened peripheral secondary immune responses by investigating the effects of immune challenges that share no antigens with the initial tumour. By utilizing preclinical mouse model systems, the many confounding factors that impact studies in patients are avoided. It has been reported that of breast tumour-bearing mice mounted weaker antibody responses and T cell proliferation in response to an immunization challenge as well as showed impaired rejection of an allogeneic tumour⁸⁴ (FIG. 3a). Similarly, our group showed that mice with AT3 breast tumours infected with *Listeria monocytogenes* mounted diminished splenic antibacterial responses marked by decreased dendritic cell expression of CD86, CD80 and CD83 at 2 days post infection²⁸ (FIG. 3b). This ultimately led to reduced CD8⁺ T cell proliferation and differentiation at 7 days post infection compared with healthy control mice infected with *L. monocytogenes*, which could be rescued by CD40 agonist treatment or surgical resection of the tumour (FIG. 3b). Strikingly, surgical tumour resection also restored humoral and cellular responses to immunization⁸⁴. Similarly, the splenic CD8⁺

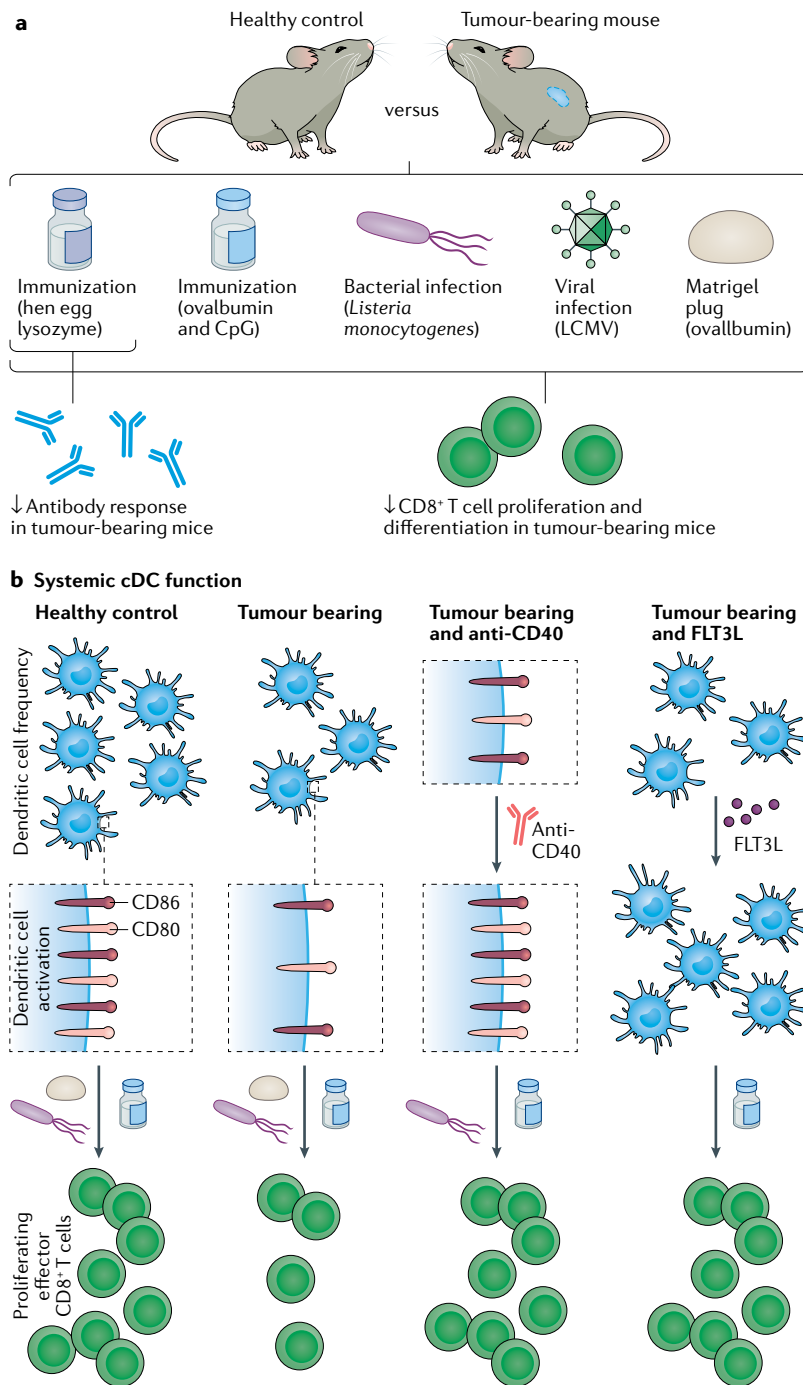


Fig. 3 | Secondary immune challenges in the context of cancer. Orthogonal challenges that do not share antigens with the tumour and drive immune responses away from the tumour microenvironment have revealed functional impairments in tumour-burdened hosts. **a** | Various challenges in tumour-bearing mice, including immunizations, bacterial and viral infections, and a Matrigel plug containing a new antigen not expressed in the tumour have shown blunted CD8⁺ T cell proliferation and differentiation. An impaired antibody response has also been observed in response to immunization. **b** | Mechanistically, several of these challenges (immunization, bacterial infection and Matrigel plug) have been linked to systemic dendritic cell paucity or impaired activation, described in the draining lymph node (dLN) and spleen. The precise drivers of dendritic cell impairment in cancer are still being investigated, but they involve altered dendritic cell development and apoptosis induced by increased circulating IL-6. Immunotherapeutic interventions that activate dendritic cells (anti-CD40) or increase their abundance (FLT3L) have restored CD8⁺ T cell proliferation and differentiation. cDC, conventional dendritic cell; LCMV, lymphocytic choriomeningitis virus.

T cell response to *L. monocytogenes* in Pan02 pancreatic tumour-bearing mice also showed perturbed T cell differentiation characterized by the acquisition of an exhausted fate¹²⁶. Suppressed splenic expansion of CD8⁺ T cells has also been observed in response to lymphocytic choriomeningitis virus in mice with pre-existing B16 melanoma¹²⁷ or AT3 breast tumours²⁸ when compared with healthy control infected mice. Vaccination of pancreatic tumour-bearing mice with ovalbumin (OVA) and CpG also led to impaired OVA-specific CD8⁺ T cell proliferation and differentiation in the spleen when compared with healthy control vaccinated mice³⁹. This impairment was linked to dendritic cell dysfunction and could be rescued by combined treatment with FLT3L and CD40 agonism to increase both dendritic cell numbers and activation, respectively³⁹ (FIG. 3b). In a PyMT-B6 mouse model of breast cancer, a Matrigel plug containing poly I:C and OVA was used as an immunogenic secondary challenge without shared antigens to the primary tumour. Pre-existing malignancy drove significantly decreased frequency of cDC1s within the plug and the dLN, which then led to a reduced number of OVA-specific CD8⁺ T cells infiltrating the plug when compared with healthy control challenged mice¹⁸. Taken together, the results of these studies show that the innate and adaptive arms of immune responses, and specifically dendritic cell and CD8⁺ T cell interactions, do not proceed optimally in the context of cancer (FIG. 3). Thus, therapeutic strategies aiming to stimulate new CD8⁺ T cell responses must overcome these obstacles.

Systemic immune biomarkers for cancer

Despite significant interest in the development of predictive biomarkers leveraging the systemic immune system, the vast majority of immunotherapy clinical trials are still performed without the use of a biomarker to guide inclusion¹²⁸. Currently, there is no systemic immune biomarker that is sufficiently established to permit bedside decision-making, although some immunological features in the TME have been shown to be associated with prognosis in various contexts. Therefore, an opportunity exists for immune biomarkers from peripheral blood to help guide patient treatment decisions.

Circulating protein biomarkers. Quantification of circulating proteins in the serum or plasma is routinely performed in various pathological contexts, and thus several studies have examined the potential of this approach to develop predictive biomarkers for cancer therapy (TABLE 2). In general, higher levels of soluble factors associated with ongoing immune responses appear to indicate improved prognosis. Increased levels of IL-2 and decreased levels of IL-6 and TNF in the blood at baseline as well as an increase in IL-4 levels on treatment were all associated with improved response to ICIs in patients with small cell lung cancer¹²⁹. Moreover, a study of patients with NSCLC found that increased serum levels of numerous inflammatory cytokines were associated with improved response to anti-PD1 therapy and overall survival¹³⁰. By contrast, high serum levels of the neutrophil chemokine IL-8 have recently been associated with poor response to ICIs in patients with melanoma,

Table 2 | Peripheral immune biomarkers in cancer

Peripheral blood immune feature	Prognosis	Tumour type ^a	Refs
High neutrophil to lymphocyte ratio	Worse response to ICIs	Melanoma ^{M/NM} , NSCLC, renal cell carcinoma ^M	132,133, 136–138
High serum IL-8	Worse response to ICIs	Melanoma, NSCLC, SCLC ^{M/NM}	129,131
Low serum LDH	Better response to ICIs	Melanoma ^{M/NM} , NSCLC ^M	132,133
Higher relative eosinophil count	Better response to ICIs	Melanoma ^{M/NM}	132
Higher relative lymphocyte count	Better response to ICIs	Melanoma ^{M/NM}	132
Higher IL-2 at baseline	Better response to ICIs	SCLC ^{M/NM}	129
Lower IL-6 at baseline	Better response to ICIs	SCLC ^{M/NM}	129
Lower TNF at baseline	Better response to ICIs	SCLC ^{M/NM}	129
Increase IL-4 on treatment	Better response to ICIs	SCLC ^{M/NM}	129
Higher TCR repertoire diversity at baseline	Better response to ICIs	Melanoma ^M , classical Hodgkin lymphoma	115,134
Fewer M-MDSCs at baseline	Prolonged overall survival after ICI (anti-CTLA4) therapy, better response to neoantigen vaccine immunotherapy	Melanoma ^{M/NM} (anti-CTLA4), diffuse midline glioma (peptide vaccine + poly-ICLC)	139–144
More CD45RA-FOXP3 ^{hi} T _{reg} cells at baseline	Relapse after surgery	Breast cancer	53
More naive and effector T _{reg} cells at baseline	Poor response to chemotherapy (platinum based with or without anti-VEGFA)	NSCLC ^M	145
More T _{reg} cells at baseline	Improved response to ICIs (anti-CTLA4)	Melanoma ^M	140
More mature natural killer cells at baseline	Better response to ICIs (anti-PD1)	Classical Hodgkin lymphoma	115
More CD3 ⁺ CD68 ⁺ CD4 ⁺ granzyme B ⁺ at baseline	Better response to ICIs (anti-PD1)	Classical Hodgkin lymphoma	115
More CD127 ^{lo} PD1 ^{lo} CD4 ⁺ T cells after treatment	Better response to ICIs (anti-CTLA4 and GM-CSF)	Melanoma ^M	92
Proliferating and/or clonal expansion CD8 ⁺ T cells after treatment	Better response to ICIs (anti-PD1 or anti-PD1 + anti-CTLA4)	Melanoma ^M	110,146,147
More T _{reg} cells after treatment	Better response to ICIs (anti-PD1)	NSCLC	148,149
Fewer PMN-MDSCs after treatment	Better response to ICIs (anti-PD1)	NSCLC	148,149

ICI, immune checkpoint inhibitor; M-MDSC, mononuclear myeloid-derived suppressor cell; NSCLC, non-small-cell lung cancer; PMN-MDSC, polymorphonuclear myeloid-derived suppressor cell; SCLC, small-cell lung cancer; TCR, T cell receptor; T_{reg} cell, regulatory T cell; VEGFA, vascular endothelial growth factor A. ^aSuperscript ^M indicates that this observation was specifically made in metastatic disease, whereas ^{NM} indicates that the observation was specifically made in non-metastatic disease.

NSCLC and small cell lung cancer^{129,131}. This observation perhaps reflects the immunosuppressive roles that neutrophils can play in antitumour immune response. In addition, low serum LDH was found to be an indicator of response to ICIs in patients with melanoma¹³² and NSCLC¹³³, and the cellular and molecular mechanisms that underlie this association merit further

investigation. Beyond secreted factors, studies have also examined whether the repertoire of lymphocyte antigen receptors in circulation provides prognostic information. Baseline diversity of TCR repertoires was also shown to predict responses to anti-PD1 and anti-CTLA4 ICIs in metastatic melanoma and classical Hodgkin lymphoma^{115,134}.

Peripheral cellular biomarkers before treatment.

Cellular biomarkers from peripheral blood are another promising approach to improve patient stratification. Simple metrics quantified from routine complete blood counts have been shown to associate with patient prognosis in various human malignancies. Of these, the neutrophil to lymphocyte ratio has emerged as a negative prognostic indicator in patients with various individual cancer types as well as in meta analyses^{19,135}. Moreover, in patients with melanoma, NSCLC and renal cell carcinoma, response to immunotherapy with ICIs is also associated with the neutrophil to lymphocyte ratio^{132,133,136–138}. ICI response in patients with melanoma was also associated with higher relative eosinophil counts and higher relative lymphocyte counts compared with non-responders¹³². Detailed cellular analyses, generally performed by flow cytometry, have also identified specific cell populations in peripheral blood that associate with outcomes. Various studies have identified associations between circulating immunosuppressive cell subsets and response to therapy. For instance, patients with melanoma with lower levels of circulating M-MDSCs at baseline were significantly more likely to achieve prolonged overall survival following anti-CTLA4 ICI^{139–143}. Similarly, in patients with diffuse midline glioma, lower frequencies of M-MDSCs in peripheral blood predict improved response to neoantigen vaccine immunotherapy¹⁴⁴. Beyond myeloid suppressive cells, a recent study demonstrated that the baseline abundance of a subset of circulating T_{reg} cells defined as CD45RA-FOXP3^{hi} were predictive of relapse after patients with breast cancer underwent surgery⁵³. High levels of circulating naive and effector T_{reg} cells in patients with NSCLC were also associated with poor response to chemotherapy¹⁴⁵. Conversely, in the context of immunotherapy, one study found that higher levels of circulating T_{reg} cells predicted improved response to anti-CTLA4 ICI in melanoma¹⁴⁰. On the other hand, cytotoxic cell subsets in the periphery have been shown to associate with improved response. The abundances of mature NK cells and a subset of cells defined by co-expression of CD68, CD4 and granzyme B were found to associate with response to anti-PD1 therapy in classical Hodgkin lymphoma patients¹¹⁵. Therefore, although there remains no systemic immune biomarker that is widely used to guide patient treatment, recent progress in this area has been quite encouraging.

Peripheral cellular biomarkers on treatment.

In addition to baseline indicators of patient outcome, various recent reports have emerged indicating that several features of immune cells in peripheral blood of patients with cancer early after immunotherapy are indicative of good outcomes. A pattern has emerged indicating that

Ovalbumin

(OVA). A model antigen derived from chicken egg whites used to study antigen-specific T cell and B cell responses in mice.

early signs of activated or proliferating lymphocytes are associated with an improved likelihood of response. A study from our group identified circulating CD4⁺ T cells with low expression of both CD127 and PD1 associated with response to anti-CTLA4 and GM-CSF in patients with melanoma⁹². In addition, various recent studies have also shown that CD8⁺ T cell proliferation and expansion in peripheral blood is associated with response to ICIs. In melanoma, the ratio of peripheral T cell proliferation to tumour burden was shown to be associated with response to anti-PD1 therapy¹⁴⁶. In this study, the peak of CD8⁺ T cell proliferation occurred after one or two cycles of therapy (3 or 6 weeks), and proliferating cells (identified as Ki67⁺) were enriched for PD1 expression. Building on this finding, several groups recently demonstrated that the expansion of specific T cell clones in peripheral blood of patients with cancer early after ICI therapy were associated with clinical responses. Clonal expansion of effector memory-like CD8⁺ T cells in peripheral blood followed by tumour infiltration was associated with response to ICIs in patients with melanoma^{110,147}. Moreover, patients across several malignancies with evidence of clonal expansion by gene expression analysis experienced greater progression-free survival when treated with anti-PDL1 therapy¹⁰⁹. A study of NSCLC has also found that an increase in circulating T_{reg} cells after treatment with anti-PD1 leads to a favourable response to treatment, whereas circulating PMN-MDSC frequencies decreased in responders^{148,149}. Thus, a series of recent studies have identified on-treatment biomarkers captured in peripheral blood analyses that both support the importance of systemic immune responses in immunotherapy and provide opportunities for improving patient care through immune monitoring.

Conclusion and future perspectives

The widespread adoption of high-throughput, high-dimensional, single-cell technologies has led to many important discoveries and atlases of diverse tumour immune microenvironments at steady state and with therapy^{150–155}. The vast majority of these studies have focused on the tumour itself rather than assessing how the global immune macroenvironment is altered compared with healthy individuals or how the peripheral immune landscape changes in response to therapy. A complete understanding of cancer and the host immune responses across diverse tumour types, patient populations and therapies requires detailed understanding not only of the TME but also of the macro-environmental alterations in immune organization. Unbiased single-cell technologies measuring the transcriptome, epigenome and proteome as well as multiple modalities simultaneously will play an integral role in the construction of comprehensive organism-scale reference maps of the immune system in cancer and of the impact of various cancer therapies. Establishing distinct types of peripheral immune organization in patients with cancer will aid personalized medicine efforts by informing the context in which therapeutic interventions will be introduced. Such studies in the context of therapy will also inform how the peripheral

immune response is regulated and dysregulated during effective or ineffective immune responses. Although many alterations to immune organization have been observed in the periphery of individuals burdened with tumours, the mechanisms driving many of these features remain unknown. Thus, future studies will also need to provide mechanistic insights into how peripheral immune reorganization is driven in order to enable the design of therapeutic strategies that restore a disrupted immune system to a healthy homeostatic immune set point. Our group found that surgical resection or blockade of specific cytokines in multiple tumour models restored many peripheral immune perturbations, suggesting that the tumour immune macroenvironment is remarkably plastic²⁸. Pairing single-cell measurements from the tumour and periphery may facilitate the identification of simplified biomarkers that can be easily sampled through blood draws and provide important clinical information to help guide treatment decisions^{53,115,134,156}.

Beyond the reorganization of the immune system in cancer, accumulating evidence also indicates that the tumour-burdened immune state does not function in the same way as an unperturbed immune system. The development of de novo antitumour immune responses orchestrated from the periphery are critical for immunotherapeutic efficacy. Therefore, any functional abnormalities within a tumour-burdened immune system may lead to suboptimal immunotherapeutic efficacy. We propose that an important avenue of future research is the identification of emergent functional properties of the tumour-burdened immune state. A growing body of evidence suggests that systemic dendritic cell dysfunction is a cause of blunted CD8⁺ T cell proliferation and differentiation in the context of cancer. Although dendritic cell-focused treatments such as anti-CD40 agonist immunotherapy^{28,39,157}, poly-ICLC¹⁵⁸ and FLT3L^{39,159} can overcome impaired dendritic cell function and paucity, the precise mechanisms underlying the dendritic cell dysfunction remain incompletely understood. As dendritic cells are the most important cell type for initiating T cell responses in cancer, deciphering why dendritic cells are functionally impaired in the periphery of individuals burdened with tumours is imperative. To date, studies have evaluated the capacity of tumour-bearing mice to mount type 1 immune responses. As the immune system can be engaged in substantially different ways based on the context of the challenge, an intriguing next step is to drive functionally distinct immune responses in tumour-burdened mice such as parasite and allergen challenges. Diverse functional challenges will elucidate emergent functional immunological alterations that are undetectable by simply examining cell population frequencies and phenotypes. Importantly, diverse functional challenges may also reveal aspects of immunity that are not perturbed by the tumour burden and, thus, inform therapeutic interventions that utilize unperturbed aspects of the immune system. Another important strategy is to design studies to thoroughly evaluate human immune responses to new challenges in patients with cancer who are treatment naive. Detailed single-cell analysis

of vaccine responses in patients with cancer as well as longitudinal monitoring of patients with high-risk mutations or organoid systems could be utilized to define the functionality of the tumour-burdened immune state in humans. These types of studies are an important step towards identifying weaknesses, or perhaps new strengths, in immune function that can rationally inform the design and implementation of new cancer immunotherapies. Additionally, as patients with cancer represent

a susceptible population for infection, these studies can inform vaccination formulations and therapeutic interventions to protect this vulnerable patient population from secondary infections. Altogether, an improved understanding of how the peripheral immune landscape is perturbed and contributes to tumour control will provide essential next steps for the field.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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