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Impact of oncogenic pathways on evasion of antitumour immune responses

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Abstract

Immunotherapeutic interventions are showing effectiveness across a wide range of cancer types, but only a subset of patients shows clinical response to therapy. Responsiveness to checkpoint blockade immunotherapy is favoured by the presence of a local, $CD8^+$ T cell-based immune response within the tumour microenvironment. As molecular analyses of tumours containing or lacking a productive $CD8^+$ T cell infiltrate are being pursued, increasing evidence is indicating that activation of oncogenic pathways in tumour cells can impair induction or execution of a local antitumour immune response. This Review summarizes our current knowledge of the influence of oncogenic effects on evasion of antitumour immunity.

Immunotherapy has emerged as an important therapeutic modality for a broad range of cancer types. Antibodies targeting the T cell inhibitory receptors cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed death receptor 1 (PD1) (or its ligand PDL1) are the most clinically advanced, with US Food and Drug Administration (FDA) approvals in melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma, bladder cancer, Hodgkin lymphoma, head and neck cancer, Merkel cell carcinoma, microsatellite instable (MSI)-high tumours, hepatocellular carcinoma and gastro-oesophageal junction cancer[1–4]. Despite these exciting advances and broad applicability of the drugs, only a minority of patients with cancer benefit from these therapies[3,5]. Analysis of pretreatment tumour biopsy samples from patients treated with checkpoint blockade therapy and/or with therapeutic cancer vaccines has revealed that patients with a pre-existing local $CD8^+$ T cell infiltrate (T cell-inflamed) were more likely to show a clinical response (data presented at the 2007 American Society of Clinical Oncology (ASCO) Annual Meeting[6] and in REFS 2,5,7–10). Approximately 35% of metastatic melanoma lesions have gene expression profiles indicative

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Author contributions

S.S. and T.F.G. researched data for the article, made substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission.

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of a T cell-inflamed tumour microenvironment (TME) phenotype. These profiles are present in a subset of patients of most cancer types, as shown by analysis of The Cancer Genome Atlas (TCGA) data[11–13]. These baseline biomarker data are consistent with the notion that checkpoint blockade therapy (in particular anti-PD1) is efficacious largely through reactivation of tumour antigen-specific T cells already present within the TME[5,14]. However, the majority of solid tumours do not show evidence of a productive T cell infiltrate and can be characterized as non-T cell-inflamed[11,15].

Mechanistic studies into the events that trigger spontaneous antitumour T cell priming have highlighted critical steps. Initial $CD8^+$ T cell priming against tumour-associated antigens occurs via basic leucine zipper transcriptional factor ATF-like 3 lineage dendritic cells (BATF3 DCs), which are capable of cross-presenting tumour-derived antigens through the class I major histo-compatibility complex (MHC) pathway[16]. Mice deficient for BATF3 DCs fail to prime tumour-specific $CD8^+$ T cells *in vivo* and fail to control growth of immunogenic tumour variants[17,18]. Studies in syngeneic tumour mouse models have indicated that tumour-derived DNA activates the stimulator of interferon genes (STING) pathway in dendritic cells (DCs) which, in turn, induces a type I interferon (IFN) cascade, both of which are important for optimal T cell priming *in vivo*[19–22]. In line with these findings, studies in human tumours have found that the presence of BATF3 DCs and induction of type I IFN correlate with T cell infiltration[23–25]. Following $CD8^+$ T cell activation in the tumour-draining lymph node, $CD8^+$ effector T cells traffic back to the inflamed TME in a CXC-chemokine receptor 3 (CXCR3)-dependent response to the chemokines CXC-chemokine ligand 9 (CXCL9) and CXCL10 (REF. 26) (FIG. 1). Notably, gene expression patterns indicative of each of these stages of productive antitumour immunity are characteristic of the T cell-inflamed TME[8,13,15,23,25,27,28].

On the basis of this working model, one might envision that the non-T cell-inflamed TME might result from a block in one or more steps in this cascade of events. Non-T cell-inflamed tumours appear to possess a number of antigens similar to that of their T cell-inflamed counterparts, thereby excluding reduced antigenicity as a predominant evasion mechanism[11,29]. Similarly, T cell-inflamed and non-T cell-inflamed tumour lesions can be present within the same patient, suggesting that in some cases, there are features within specific metastatic sites that might cause defective T cell infiltration[30–32]. These observations indicate that molecular alterations in specific tumour cell-intrinsic molecular pathways might affect the degree of T cell infiltration into a given tumour. Indeed, increasing clinical evidence suggests that activation of certain oncogenic pathways is associated with the non-T cell-inflamed TME and the potential for immunotherapy resistance (data presented at the American Association for Cancer Research (AACR) Annual Meeting 2017 (REF. 33) and in REFS 31,34). This Review summarizes current knowledge on how oncogenic pathways, activated through gain-of-function alterations in oncogenes or loss-of-function alterations in tumour suppressor genes, influence the local antitumour immune response and highlights potential therapeutic solutions.

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Gain-of-function alterations

Oncogenic WNT–β-catenin signalling reduces T cell recruitment.

Analysis of metastatic melanoma revealed that 34.2% (91/266 cases) of the melanoma metastatic lesions were non-T cell-inflamed. Of those non-T cell-inflamed lesions, 48% showed evidence for activation of WNT–β-catenin signalling specifically in tumour cells[13]. Similarly, analysis of *BRAF*-mutated primary melanoma lesions showed that a lack of T cell infiltration was associated with upregulation of β-catenin signalling in tumour cells and therapeutic resistance (data presented at the AACR Annual Meeting 2017 (REF. 33) and in REF. 35). To determine whether tumour cell-intrinsic oncogenic activation of β-catenin was causally related to T cell exclusion, a genetically engineered mouse model (GEMM) conditionally expressing $Braf^{V600E}$ and floxed *Pten* alleles (referred to as $Braf^{LSL-V600E}/Pten^{fl/fl}$)[36,37] was interbred with a GEMM expressing a Cre-inducible, dominant stable form of β-catenin[38]. Both β-catenin-positive and β-catenin-negative $Braf^{LSL-V600E}/Pten^{fl/fl}$ GEMMs developed melanoma with 100% penetrance. However, further analysis revealed that β-catenin-positive tumours had minimal T cell infiltration and were resistant to checkpoint blockade therapy[13,39]. Indeed, in β-catenin-positive tumours, production of CC-chemokine ligand 4 (CCL4) and other chemokines by melanoma cells was reduced, which at least partly caused a severely reduced recruitment of BATF3 DCs into the TME. This led to defective host priming of antigen-specific T cells, causing resistance to checkpoint blockade therapy[13]. Indeed, therapy resistance could be reversed by injection of mature DCs into β-catenin-positive tumours. Together, these data show that tumour cell-intrinsic activation of β-catenin signalling could contribute to a non-T cell-inflamed TME and cause resistance to checkpoint blockade immunotherapy *in vivo*[13]. Further studies revealed that the lack of effector T cell infiltration in β-catenin-positive tumours was also due to defective effector T cell trafficking, which in β-catenin-negative tumours was driven by the chemokines CXCL9 and CXCL10, produced mainly by BATF3 DCs. Thus, the absence of BATF3 DCs from β-catenin-positive tumours caused defective early T cell priming as well as defective trafficking of effector T cells into the TME[40]. This is the reason why adoptive transfer of tumour-specific effector T cells or prophylactic vaccination inhibited growth of β-catenin-negative tumours but failed to inhibit growth of β-catenin-positive tumours[40]. These data demonstrate the possibility that upregulation of β-catenin and/or defective recruitment of BATF3 DCs can potentially mediate secondary resistance to immunotherapies (FIG. 2; TABLE 1).

The WNT–β-catenin pathway appears to be associated with a non-T cell-inflamed TME in cancer types beyond melanoma, as suggested, for example, in studies on bladder cancer and head and neck cancer[41,42]. In addition, an ongoing analysis of TCGA data across all solid tumour types has indicated a similar correlation between the non-T cell-inflamed phenotype and gain-of-function of β-catenin signalling in tumour cells in the majority of tumour types included in the analysis (data presented at the 2016 ASCO Annual Meeting[43]). This includes colorectal carcinoma (CRC), which typically has a high frequency of genetic alterations linked to β-catenin pathway activation and at the same time shows rare clinical benefit of anti-PD1 immunotherapy[44]. A recent study on the immune and stromal classification of CRC and its association with CRC molecular subtypes suggested that tumours of the MSI

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subtype and the mesenchymal subtype appeared T cell-inflamed, while tumours of both the metabolic subtype and the canonical subtype appeared non-T cell-inflamed[45]. In line with this distinction, both the MSI and the mesenchymal subtypes do not rely on activation of WNT–β-catenin signalling for tumour development, whereas the canonical and the metabolic subtypes rely on inactivation of the adenomatous polyposis coli gene for tumour development[46]. Moreover, patients with CRC who have tumours of the MSI subtype have high response rates to anti-PD1 therapy[47]. It is suggested that these high response rates of MSI tumours correlate with the potential for high levels of neoantigens in these tumours[48]. However, it remains to be shown whether the lack of β-catenin pathway activation in these tumours contributes to the clinical benefit of anti-PD1 therapy. Of note, a case report from a patient with ovarian cancer described that activation of the WNT–β-catenin pathway in some metastatic lesions was associated with a lack of T cell infiltration and with a lack of response to checkpoint blockade therapy[31]. Prospective preclinical and clinical studies are ongoing to evaluate whether tumour cell-intrinsic WNT–β-catenin signalling is associated with checkpoint blockade resistance in larger cohorts of patients and whether targeting this pathway can be a potential strategy to improve immunotherapy outcomes (S.S. and T.F.G., unpublished data).

Gain of MYC function inhibits T cell activation and infiltration.

The transcription factor MYC regulates cell proliferation, differentiation and survival and is overexpressed in many cancers[49,50]. Most commonly, activation of the pathway occurs through gene amplification or constitutive expression rather than through point mutations[51]. Seminal work using a GEMM with conditionally regulated *Myc*, and in which *Myc* over-expression leads to the development of osteogenic sarcomas, showed that continuous overexpression of *Myc* was necessary to maintain tumour cell persistence, an observation known as ‘oncogene addiction’ (REF. 52). In the same model, when *Myc* was inactivated, tumour cells underwent cell cycle arrest and apoptosis, leading to improved animal survival[50]. Interestingly, subsequent work revealed that host T cells significantly contributed to tumour cell elimination following *Myc* inactivation[53]. In particular, while $CD4^+$ T cells were not required for the induction of cell cycle arrest following reactivation of *Myc*, $CD4^+$ T cells were required for the induction of senescence[53]. Mechanistically, inactivation of *Myc* led to a marked decrease in the expression of PDL1, the dominant ligand for the inhibitory receptor PD1 on activated T cells, and leukocyte surface antigen CD47, which limits activation of antigenpresenting cells (APCs) through binding to signal regulator protein-α (SIRPα) on macrophages and DCs and blocking antigen uptake[54,55]. PDL1 and CD47 transcription was controlled directly by MYC, and restoring CD47 or PDL1 expression by transfection reversed the pheno-type and prevented accumulation of tumour-associated T cells[56] (FIG. 2; TABLE 1). Restoring PDL1 or CD47 also led to stabilization of $CD31^+$ microvessels and expression of the pro-angiogenic molecules tyrosine-protein kinase receptor TIE2 (also known as TEK) and angiotensin 1 (ANG1) in *Myc*-inactivated tumours[56]. The connection between an active immune response and vessel stabilization should be further investigated as functional vasculature is important for extravasation of lymphocytes[26,56].

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Loss-of-function alterations

Loss of LKB1 function decreases T cell infiltration.

In addition to immune evasion mechanisms linked to defective T cell priming, some tumour-intrinsic pathways may mediate immune evasion through the recruitment of immune inhibitory cell populations. A key example is liver kinase B1 (*LKB1*; also known as *STK11*), a tumour suppressor gene that is mutated in approximately 30% of patients with NSCLC and has been associated with worse prognosis[57]. Analysis of the TME in a *Kras*-driven NSCLC GEMM engineered either with or without *Lkb1* deletion revealed a marked increase in tumour-associated neutrophils in *Lkb1*-deficient tumours along with a modest decrease in the quantity of infiltrating T cells[58]. Phenotypic analysis of tumour-infiltrating lymphocytes (TILs) revealed decreased cytokine production and increased expression of PD1, hepatitis A virus cellular receptor 2 (HAVCR2; also known as TIM3) and lymphocyte activation gene 3 protein (LAG3), which is consistent with a dysfunctional TIL state. Differential gene expression profiling indicated that interleukin-33 (IL-33), CXCL7 and IL-6 were expressed at higher levels by *Lkb1*-deficient tumour cells. Functionally, depletion of neutrophils or blockade of IL-6 resulted in increased T cell infiltration into *Lkb1*-deficient tumours and improved efficacy of anti-PD1 antibody therapy[58] (FIG. 2; TABLE 1).

The mechanisms by which IL-6 production is regulated and how it interferes with antitumour immunity downstream are incompletely understood. Increased activation of the signal transducer and activator of transcription 3 (STAT3) signalling pathway was observed in *Lkb1*-deficient NSCLC tumours, which provides one potential link[58]. Known downstream pathways of LKB1 include the AMP-activated protein kinase (AMPK) and mTOR pathways, downstream targets of which include cyclin D1 (*CCND1), MYC* and hypoxia-inducible factor 1-alpha (*HIF1A*)[59]. Activation of some of these downstream factors is associated with an immune inhibitory phenotype in other cancer model systems (for examples, see MYC gain-of-function and other oncogenic pathways described above) and is worth investigating mechanistically in future experiments[56].

Loss of PTEN reduces efficient T cell priming.

Inactivating mutations or deletion of *PTEN* has been associated with defective T cell infiltration in melanomas[60]. Interestingly, in tumours showing partial PTEN loss resulting in intratumoural heterogeneity, T cells were found to localize only to regions that retained PTEN expression[60]. In a mouse preclinical model, a PI3Kβ isoform-preferential inhibitor was therapeutically synergistic with immunotherapy *in vivo*, arguing that selective PI3K inhibitors might have the potential to augment immunotherapy effectiveness in the clinic as well. A separate study found that PI3K inhibitors could improve anti-PD1 efficacy in a clinically relevant breast cancer mouse model[61]. In patients with melanoma who were treated with an anti-PD1 monoclonal antibody (mAb), clinical response was favoured in patients showing high PTEN expression in the tumour. Together, these data suggest that mutations or deletion of *PTEN* represent an immune evasion mechanism. A more recent study has linked deletion of specific regions of chromo-some 10 that flank the *PTEN* gene with failed response to checkpoint blockade therapy[25].

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The mechanism by which PTEN deletion and/or PI3K activation might promote immune evasion is incompletely understood and could be multi-factorial. Gene expression profiling revealed that CCL2 and vascular endothelial growth factor (VEGF) were elevated in melanoma patient samples harbouring *PTEN* loss, while expression of MHCI and PDL1 was unaffected[60]. In addition, a decrease in the expression of autophagy-related genes was observed. This decrease in gene expression was accompanied by reduced auto-phagic activity in melanomas with *PTEN* loss and in human melanoma cell lines with *PTEN* knockdown[60].

This finding is of interest because autophagy in cancer cells has been reported to contribute to T cell priming by increasing DC activation and cross-presentation following tumour implantation in mice[62–64]. In particular, auto-phagy was required for ATP release from tumour cells undergoing death in response to chemotherapy, thereby enabling ATP-dependent DC recruitment[64]. In addition, tumour cell-derived autophagosomes are able to trigger an immune response by delivering neoantigens to APCs[62,63]. *PTEN*-deficient melanoma cells were also more resistant to $CD8^+$ T cell-mediated killing *in vitro*[60] (FIG. 2). Importantly, when autophagy was induced in these cells by overexpressing the gene microtubule-associated protein 1 light chain 3 beta (*MAP1LC3B*), which encodes an important autophagosome protein, the susceptibility of *PTEN*-deficient melanoma cells to T cell-mediated killing was restored[60]. It remains to be investigated whether the reduced autophagic activity in *PTEN*-deficient tumour cells is associated with a decrease in type I IFN induction and decreased T cell priming in the tumour-draining lymph node. Regardless of this critical mechanistic gap, the studies in melanomas with PTEN loss[60] have motivated clinical exploration of specific PI3K inhibitors in combination with checkpoint blockade immunotherapy[65].

Loss of p53 function decreases T cell infiltration.

Inactivating mutations of the tumour suppressor gene *Trp53* have been associated with reduced immune infiltration[66]. This study used an orthotopic tumour model in which a *Hras*-mutant mouse hepatoblast cell line was engineered to express a doxycycline-inducible, *Trp53* targeting microRNA. Here, the growth of hepatocarcinoma induced by *Trp53* silencing could be inhibited upon doxycycline withdrawal and restoration of *Trp53* expression[66]. Interestingly, gene expression profiling revealed that tumour regression was associated with increased expression of immune genes in the tumour cells. These genes included colony stimulating factor 1 (*CSF1*), *CCL2*, *CXCL1* and *IL-15* along with the adhesion molecules intercellular adhesion molecule 1 (*ICAM1*) and vascular cell adhesion molecule 1 (*VCAM1*)[66]. In an independent study using the same model, induction of *Trp53* resulted in increased expression of CCL2, CCL3, CCL4, CCL5, CXCL1 and CXCL2 as well as the cytokines IL-1β, IL-12β and IL-15 (REF. 67). In this model, tumour regression was mediated by natural killer (NK) cells and the membrane protein NKG2D expression rather than by T cell infiltration, arguing that oncogenic pathways might also influence innate lymphoid-like cells (FIG. 2; TABLE 1).

A recent analysis of human tumours has also suggested a correlation between p53 status and immune cell infiltrates[68]. Within a subset of basal-like breast cancer samples, a correlation

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between loss of p53 function and absence of a T cell gene signature was observed. Patients with loss of heterozygosity as well as a p53 mutation showed the most profound decrease in T cell infiltration[68]. Within an oestrogen receptor (ER)-negative cohort, similar results were observed, while ER-positive patients with breast cancer did not show any correlation between p53 loss and lack of T cell infiltration[68]. It will be interesting to investigate a range of additional tumour types to uncover similar associations in other cancers. Given the wide range of changes in chemokine production associated with dysregulation of the p53 pathway, additional studies will be needed to investigate which immune cell types are affected in patients with distinct types of cancer.

Impact of other oncogenic pathways

Early data have suggested the potential for additional oncogenic pathways to affect antitumour immunity. Recent findings in patients with gliomas as well as in a preclinical glioma mouse model have provided evidence that mutations in the genes encoding isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are associated with reduced T cell infiltration[69]. Initial investigation of samples from the TCGA database provided evidence that tumours with activating mutations in *IDH1* or *IDH2* show reduced expression of cytotoxic T cell markers, including *CD8A* and *IFNG*. Further analysis using an orthotopic model of glioma showed that tumour cells engineered to have mutated *Idh1*, leading to increased IDH1 activity, showed diminished recruitment of T cells. Mechanistically, increased IDH1 activity led to reduced levels of STAT1 protein as well as reduced phosphorylated STAT1. Diminished STAT1 signalling in glioma cells was associated with reduced expression of CXCL10, a critical chemokine for effector T cell recruitment. Finally, exposure of tumour cells to an IDH1 inhibitor restored STAT1 signalling, CXCL10 expression and T cell infiltration *in vivo*[69]. The fact that IDH1 inhibitors are currently in clinical development raises the possibility of evaluating whether immune cell infiltration is augmented with these agents in human cancer patients[70]. The role of tumour cell-intrinsic tyrosine-protein kinase JAK1–STAT1 signalling for immune-mediated tumour control is in keeping with analysis of tumours isolated from patients with secondary resistance to anti-PD1 therapy, in which mutations in the IFNγ signalling pathway were observed in two cases. However, whether there is a direct connection between JAK1–STAT1 signalling and anti-PD1 resistance and whether there is crosstalk with tumour oncogenic pathways remain unclear[24,25,71].

In contrast to the decreased STAT1 signalling described above, STAT3 signalling is frequently activated in human tumours[72]. *In vitro* studies have indicated that inhibition of STAT3 can generate a pro-inflammatory milieu, whereas augmentation of STAT3 signalling can decrease expression of pro-inflammatory mediators, including the chemokines CCL5 and CXCL10 (REFS 73,74). This concept was supported *in vivo* using a genetically induced prostate cancer model, as well as in a model of carcinogen-induced NSCLC, indicating that oncogenic STAT3 signalling could modulate the immuno biology of the TME[75,76]. In this prostate cancer model driven by *Pten* loss, tumour cells underwent senescence and showed an increase in phosphorylated STAT3 (REF. 75). Transcriptional activity by STAT3 induced the expression of chemokines and cytokines, including CXCL1, CXCL2 and macrophage CSF (MCSF), associated with the recruitment of myeloid-derived suppressor cells (MDSCs). Ablation or inhibition of STAT3 signalling eliminated MDSC accumulation and

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restored T cell accumulation within the tumour[75]. Whether alterations in STAT3 signalling and loss of PTEN function are frequently intertwined should be investigated in future studies.

Studies in bladder cancer have pointed to additional candidate oncogenic pathways linked to immune exclusion. As in melanoma, β-catenin pathway activation was found in tumours within a non-T cell-inflamed subset of bladder cancers. In addition, tumours of this non-T cell-inflamed subset also contained fibroblast growth factor receptor 3 (FGFR3)-activating mutations[41] or evidence for peroxisome proliferator-activated receptor-γ (PPARγ)-signalling activation[41]. A separate study has shown that bladder cancers can be subgrouped into those expressing high levels of claudin and those expressing low levels of claudin, which can be linked to the level of immune response[77]. Interestingly, tumours of the claudin-low subset expressed immune gene signatures at high levels as well as genes consistent with active immunosuppression and had decreased frequencies of *FGFR3* mutations and *PPARG* amplification[77], a finding in line with the observations described above regarding non-T cell-inflamed bladder cancers. In addition, PPARγ-signalling activation in bladder cancer has recently been linked to mutations in the nuclear hormone receptor gene retinoid × receptor-alpha (*RXRA*), which encodes a cofactor of PPARγ[78]. These findings suggest the possibility of pharmacologic targeting of this pathway as a strategy to improve immune cell infiltration.

Nuclear factor-κB (NF-κB) signalling has a variety of effects on tumour development and on the inter action with host stromal cells. For example, activation of the NF-κB signalling pathway in tumour cells promotes tumorigenesis in inflammation-induced carcinogenesis models[79,80]. In other settings, NF-κB signalling might be an additional candidate oncogenic pathway that locally inhibits host immune responses. In inflammation-induced cancer models, including CRC models, activation of NF-κB signalling has indeed been associated with increased inflammation[81], though by an extrinsic mechanism mediated through exogenous cytokines, such as tumour necrosis factor (TNF)[82]. Constitutive activation of NF-κB within tumour cells also has been reported to increase the expression of specific chemokines in a transplantable colon cancer model, which could potentially promote the immune response[81,83]. In contrast to those studies, inhibition of NF-κB signalling in lung adenocarcinoma cells resulted in increased chemokine production[84]. The frequent activation of NF-κB signalling in human cancers warrants further investigation into the potential impact on local antitumour T cell responses, for example, by using GEMMs. The overall effect of oncogenic NF-κB might depend on the cellular context and the type of immune cells involved, either tumour-promoting inflammatory cells or antitumour adaptive immune cells.

Several of the above-mentioned oncogenic pathways are intertwined with changes in oxygen availability and tumour cell metabolism. Recent studies in ovarian and breast cancer have highlighted the impact of VEGF and cyclooxygenase 1 (COX1) and COX2 expression on the local antitumour immune response[85,86]. These studies provide evidence that hypoxia-mediated VEGF increased FAS-ligand (FASL) expression on endothelial cells, in a COX1-dependent manner, and thus induces T cell death during extravasation[85]. Similarly, hypoxia-induced prostaglandin E2 (PGE2) and/or COX2 stress responses result in an unproductive

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IL-6-driven inflammatory response. Here, acetylsalicylic acid-mediated inhibition of COX2 was found to be sufficient to a switch to an IFNγ-driven antitumour response[86]. Similar to other oncogenic pathways, hypoxia is also associated with a reduction in recruitment and activation of BATF3 DCs, which would provide a mechanistic link to poor T cell infiltration[86]. Despite these interesting links between hypoxia and immune evasion, it remains unclear whether specific targetable oncogenic pathways are responsible for these processes. Many oncogenic pathways, such as the MYC pathway, regulate metabolism in tumour tissue, which could potentially affect nutrient availability and enforce dysfunctional cellular metabolism in infiltrating immune cells, thereby affecting the immune response[87]. For example, T cells increase their glycolytic flux upon activation, which might be impaired when glucose as fuel is limited[87]. In addition, HIF1 regulates a number of metabolic pathways and could affect immune cell metabolism and activity in the tumour[87]. However, whether metabolic dysregulation is directly linked to the degree of T cell infiltration in the tumour remains to be shown.

Lastly, epigenetic mechanisms may play a role in differential tumour cell gene expression and thus immune cell infiltration. One example of such a mechanism is the regulation of chemokine gene expression in human ovarian cancer[88]. The gene loci for the chemokines CXCL9 and CXCL10 were found to be epigenetically silenced in ovarian tumour cells, which was associated with decreased effector T cell recruitment. It is conceivable that expression of additional immunologically relevant genes might be similarly affected by epigenetic mechanisms, which remains to be investigated. If such a regulation is present, it would suggest that histone deacetylase inhibitors and/or DNA methyltransferase inhibitors have the potential to augment adaptive immunity within the TME and expand the effectiveness of cancer immunotherapies.

Conclusions and outlook

T cell infiltration into the TME is associated with an increased likelihood of immunotherapy efficacy, including checkpoint blockade therapy. As such, understanding the molecular mechanisms that contribute to a reduction of effector T cell infiltration should enable the development of novel agents that restore T cell infiltration and promote immunotherapy efficacy. Several of the biochemical pathways identified to date that are linked to T cell exclusion are candidates for molecularly targeted therapy. For example, PI3K inhibitors are being investigated for *PTEN*-deficient tumours[65]. Because of the fact that PI3K is also involved in T cell activation, thoughtful dose and schedule regimens may have to be considered. Alternatively, isoform-specific PI3K inhibitors that favour tumour cell-intrinsic activity with relative sparing of T cell functionality could be considered. As a general drug development paradigm, once a tolerable dose and schedule are obtained, then patients selected on the basis of expression of the relevant target in the tumour could be analysed for T cell infiltration. In addition, immune gene expression profiling on biopsy samples before and after treatment with the targeted agent could be carried out. It is interesting to consider that these drugs as single agents do not necessarily have to demonstrate clinical activity *per se*. Rather, once a given drug evidently promotes a T cell-inflamed TME, combination studies with immunotherapies such as anti-PD1 or anti-PDL1 mAbs could be rapidly developed. Some identified pathways, such as the WNT–β-catenin pathway, have been

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challenging to inhibit in a global fashion as the pathway has critical importance in normal tissues, such as bone and the gastrointestinal tract. Thus, new screens for agents that selectively restore the relevant immune gene targets while sparing global shutdown of certain pathways could be considered.

An alternative approach to promote *de novo* T cell activation and immune infiltration into the TME of non-T cell-inflamed tumours is to leapfrog over the array of altered oncogenic pathways and restore the earliest rate-limiting step that appears to be absent from those tumours in general — that is, by restoring recruitment and activation of BATF3 DCs. For example, direct intratumoural injection of activated BATF3 DCs has restored T cell infiltration in the β-catenin-positive $Braf^{LSL-V600E}/Pten^{fl/fl}$ GEMM[13,40]. The activation of APCs using agonists of the cyclic GMP-AMP synthase (cGAS)–STING pathway is also being considered, and the first STING agonist is currently in phase I clinical development[20,89]. The early signs of success using intratumoural injection of oncolytic viruses, such as talimogene laherparepvec (T-Vec), which is FDA approved for melanoma[90], as well as additional similar approaches with engineered Coxsackie viruses, also indicate the potential of engaging innate immune pathways that could in principle promote T cell priming and infiltration into ‘cold’ tumours, which are not responsive to immunotherapy[91]. An additional important consideration is intensifying the analysis of patients who develop secondary resistance following initial clinical response to immunothera-pies, such as anti-PD1. Re-analysis of progressing lesions following initial clinical response should determine whether the selection for subsets of tumour cells with active immune-evasive oncogenic pathways might lead to secondary resistance as well.

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Checkpoint blockade therapy

Includes all therapies targeting immune inhibitory molecules or pathways mediating a decrease of T cell function within the tumour microenvironment. The most prominent examples are anticytotoxic T lymphocyte antigen 4 (CTLA4) and anti-programmed death receptor 1 (PD1) antibodies.

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T cell-inflamed

A microenvironment in which $CD8^+$ T cells are found within the tumour mass or the invasive margin of the tumour. T cells produce interferon γ (IFNγ) and other cytokines yet at the same time express immune inhibitory molecules on their surface, including programmed death receptor 1 (PD1).

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Non-T cell-inflamed

A microenvironment that is representative of all tumour microenvironments with no evidence of an ongoing $CD8^+$ T cell-driven immune response and lack of expression of key chemokines and cytokines. This group of tumours might be quite diverse.

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Basic leucine zipper transcriptional factor ATF-like 3 lineage dendritic cells

(BATF3 DCs). Cells defined by the expression of the transcription factors BATF3 and interferon regulatory factor 8 (IRF8). In mice, they express lineage markers CD8α and/or CD103 (also known as ITGAE), while in humans, they express thrombomodulin (TM; also known as CD141). This lineage of DCs has the capability to cross-present tumour-derived antigens to $CD8^+$ T cells.

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Oncogenic pathways

Tumour cell-intrinsic signalling pathways with a known capability to mediate tumour induction or progression from within the tumour cells themselves. They are often but not always associated with specific mutations in oncogenes or tumour suppressor genes.

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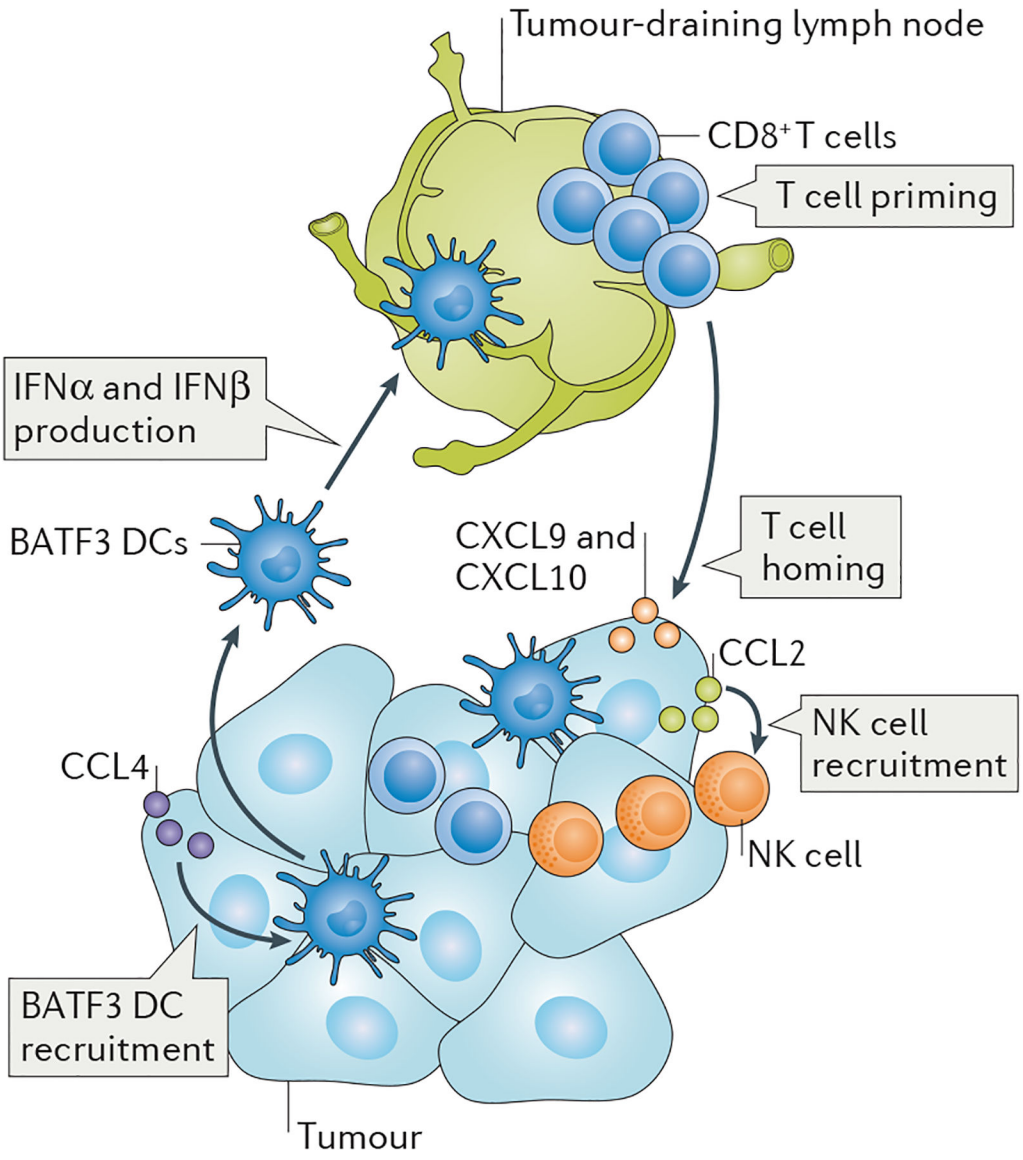

Figure 1 |. Induction phase of a productive antitumour immune response.

From left to right: tumour cells produce CC-chemokine ligand 4 (CCL4), inducing the recruitment of basic leucine zipper transcriptional factor ATF-like 3 lineage dendritic cells (BATF3 DCs). BATF3 DCs sense the tumour and produce interferon α (IFNα) and IFNβ while migrating to the tumour-draining lymph node. Here, activated BATF3 DCs prime antigen-specific T cells, which subsequently home back into the tumour under the influence of CXC-chemokine ligand 9 (CXCL9) and CXCL10. Other chemokines (for example, CCL2) recruit other effector cells such as natural killer (NK) cells.

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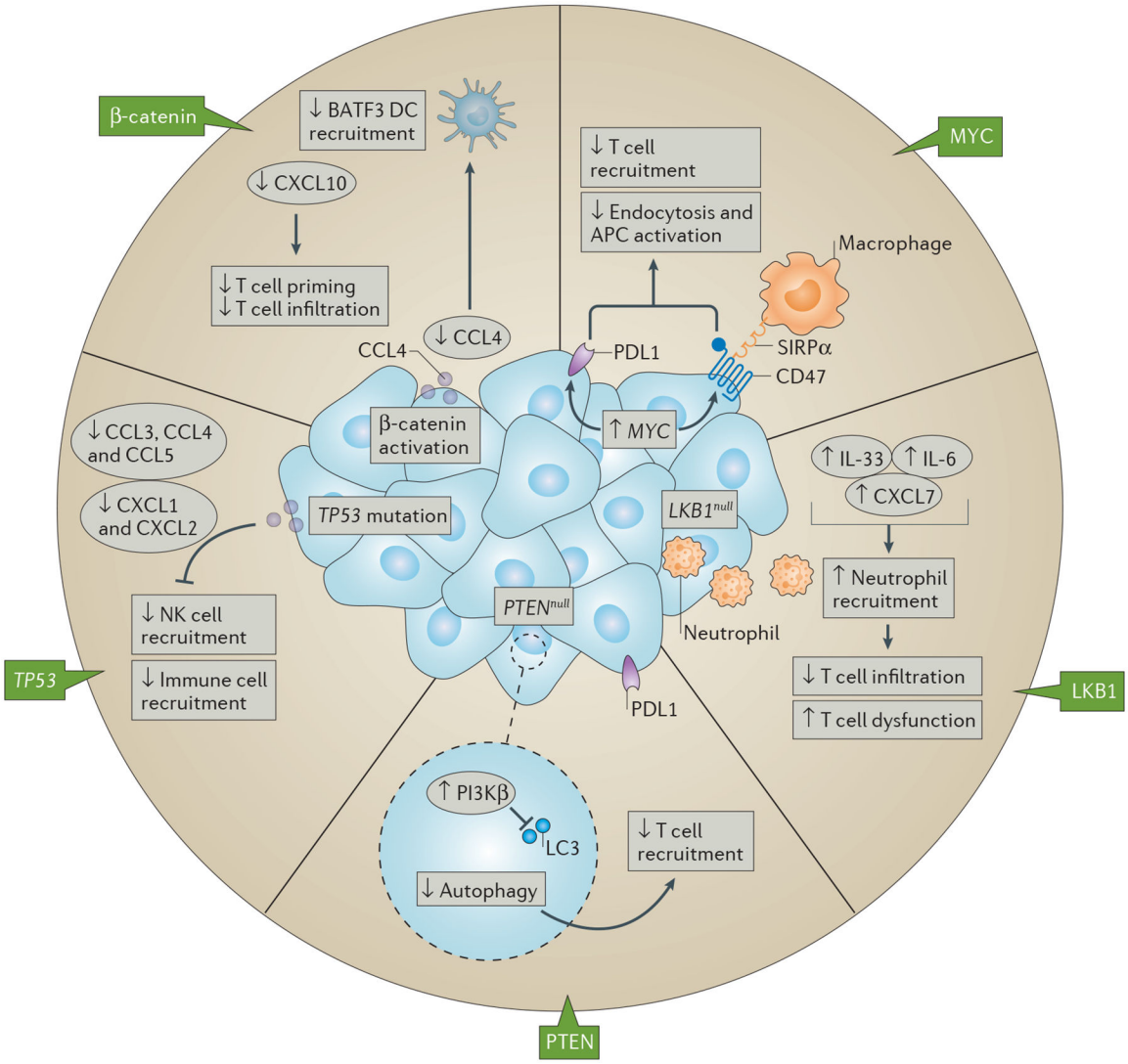

Figure 2 |. Impact of oncogenic signalling on immune inhibitory pathways and cell populations. β-catenin: immune exclusion mediated by activation of the WNT–β-catenin pathway occurs when expression of CC-chemokine ligand 4 (CCL4) is inhibited and thus basic leucine zipper transcriptional factor ATF-like 3 lineage dendritic cells (BATF3 DCs) are no longer recruited into the tumour microenvironment. Consequently, owing to a lack of CXC-chemokine ligand 10 (CXCL10) production by BATF3 DCs, no T cell priming occurs, and effector T cells are not recruited into the tumour. MYC: activation of MYC signalling enhances the expression of leukocyte surface antigen CD47 and programmed cell death 1 ligand 1 (PDL1) on tumour cells through transcriptional regulation. Expression of these immune inhibitory molecules interferes with antigen uptake by antigen-presenting cells (APCs) via engagement with signal regulator protein-α (SIRPα) and inhibits T cell function via PD1 engagement, respectively. LKB1: loss of liver kinase B1 (LKB1) signalling within tumour cells results in increased expression of various cytokines, including interleukin-6 (IL-6), IL-33 and CXCL7. IL-6 mediates recruitment of neutrophils into the tumour microenvironment, an immunosuppressive cell type that contributes to reduced T cell

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infiltration and promotes T cell dysfunction. *LKB1* mutations can also lead to activation of MYC signalling within the same tumour cells, providing potential crosstalk between pathways. PTEN: loss of PTEN protein function and thereby activation of PI3K inhibits lipidation of the autophagosome protein LC3 and autophagy in tumour cells, a process that can diminish T cell priming and also mediate resistance to T cell-mediated apoptosis. *TP53*: effector T cell exclusion from the tumour can occur owing to inactivating *TP53* mutations, which results in reduced chemokine production by tumour cells. In particular, *TP53*-mutated tumour cells lack production of key chemokines required for the recruitment of natural killer (NK) cells and T cells, which is required for NK cell recruitment into the tumour microenvironment.

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Table 1 |

Impact of oncogenic pathways on the local antitumour immune response

Pathway	Innate immune cells	Adaptive immune cells	Suppressive immune cell types	Studies in murine models	Studies involving human patients
WNT-p-catenin	BATF3 DCs	T cells	Not affected	14,40,41	14,32,42–45
MYC	SIRPa+ DCs; SIRPa+ macrophages	T cells	Unknown	52,53	–
LKB1	–	T cells	Neutrophils	57	56,57
PTEN (PI3K)	DCs; macrophages	T cells	Unknown	60	26,60
p53	NK cells	T cells	Unknown	64,65	66

BATF3, basic leucine zipper transcription factor lineage; DCs, dendritic cells; LKB1, liver kinase B1; NK, natural killer; SIRPα, signal regulator protein-α.