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Tumour-intrinsic resistance to immune checkpoint blockade

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Abstract

‘Immune checkpoint blockade’ for cancer describes the use of therapeutic antibodies that disrupt negative immune regulatory checkpoints and unleash pre-existing antitumour immune responses. Antibodies targeting the checkpoint molecules cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD1) and PD1 ligand 1 (PD-L1) have had early success in the clinic, which has led to approval by the US Food and Drug Administration of multiple agents in several cancer types. Yet, clinicians still have very limited tools to discriminate a priori patients who will and will not respond to treatment. This has fuelled a wave of research into the molecular mechanisms of tumour-intrinsic resistance to immune checkpoint blockade, leading to the rediscovery of biological processes critical to antitumour immunity, namely interferon signalling and antigen presentation. Other efforts have shed light on the immunological implications of canonical cancer signalling pathways, such as WNT–β-catenin signalling, cell cycle regulatory signalling, mitogen-activated protein kinase signalling and pathways activated by loss of the tumour suppressor phosphoinositide phosphatase PTEN. Here we review each of these molecular mechanisms of resistance and explore ongoing approaches to overcome resistance to

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Author contributions

The authors contributed equally to all aspects of the article.

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immune checkpoint blockade and expand the spectrum of patients who can benefit from immune checkpoint blockade.

Cancer immunotherapy is a strategy to treat malignancies by leveraging the cytotoxic potential of the human immune system, especially tumour-specific cytotoxic T cells. Among the different types of cancer immunotherapy, immune checkpoint blockade has had the broadest impact, with several antibodies targeting cytotoxic T lymphocyte antigen 4 (CTLA4) or the programmed cell death 1 (PD1)–PD1 ligand 1 (PD-L1) axis approved for use in a number of different cancers. A large number of antibodies and small molecules targeting other putative immune checkpoints (such as LAG3, TIGIT, TIM3, B7H3, CD39, CD73 and adenosine A2A receptor), disrupting negative regulation between tumour cells and T cells, or myeloid cells and T cells, are in clinical and preclinical development.

Patient-intrinsic factors (such as age, sex, HLA genotype and genetic polymorphisms), tumour stroma-intrinsic factors (such as the host immune system and tumour-associated stroma) and environmental factors (such as the gut microbiota) may contribute to the success or failure of immune checkpoint blockade[1–3]. However, tumour cell-intrinsic factors (herein defined as tumour-intrinsic factors), relating to the genetic, transcriptional or functional profile of the tumour cells themselves, are among the main determinants of response and resistance. The importance of tumour-intrinsic factors is reflected in the wide variation of response rates to immune checkpoint blockade across histological types and the high response rates of tumours with similar molecular and genetic features (for example, microsatellite instability). These tumour-intrinsic factors can also influence the involvement of some tumour cell-extrinsic factors (such as the host immune system and tumour-associated stroma) in therapy resistance.

In this Review, we focus on tumour-intrinsic factors of resistance to immune checkpoint blockade. In doing so, we revisit the immunological basis for tumour responses to immune checkpoint blockade, highlight key biomarkers and discuss how these reflect the tumour-intrinsic factors that promote responsiveness to immune checkpoint blockade. We then look at the mechanisms by which tumour-intrinsic defects can lead to resistance to immune checkpoint blockade and highlight existing and emerging approaches to overcome tumour-intrinsic mechanisms of resistance.

Tumour-intrinsic mechanisms of resistance

The factors that determine the induction and maintenance of a naturally occurring antitumour T cell response are complex. Characteristics that are intrinsic to tumour cells themselves — such as mutational landscape, function of interferon signalling pathways, expression of antigen-presenting molecules and immune-evasive oncogenic signalling pathways — influence the priming, activation and recruitment of T cells to the tumour microenvironment, which are necessary for an immune response in the context of immune checkpoint blockade. Likewise, resistance to immune checkpoint blockade can result from disruptions in any of these key tumour characteristics, either by preventing a de novo antitumour immune response or by counteracting an ongoing antitumour response.

Insufficient tumour antigenicity

Several studies have demonstrated the potential of tumour neoantigens to serve as effective targets for antitumour immunity, and there is correlation between mutational burden and response to immune checkpoint blockade across malignancies[4–6]. In a patient who responded to anti-CTLA4 immune checkpoint blockade, it was shown that T cells specific for a particular tumour neoantigen previously existed within the tumour microenvironment and expanded in response to anti-CTLA4 therapy[7]. In a mouse methylcholanthrene-induced sarcoma model, T cells specific for neoantigens expand and gain antitumour functionality in response to immune checkpoint blockade[8]. Potent neoantigen-specific T cells can even be detected within the tumour microenvironment in the absence of immune checkpoint blockade. In a patient with metastatic cholangio-carcinoma, tumour-infiltrating lymphocytes harboured a population of $CD4^+$ T cells specific for a tumour neoantigen. Adoptive transfer of enriched mutation-specific T cells resulted in an effective antitumour response[9]. The accumulating evidence that neoantigens are key cancer immunogens supports the promising early results of ongoing studies of neoantigen-based tumour vaccines[10]. The observation that patients with microsatellite instability due to mismatch repair defects have high response rates to immune checkpoint blockade further supports the role of neoantigens in the antitumour immune response. Conversely, tumours with poor antigenicity are less likely to harbour intrinsic sensitivity to immune checkpoint blockade.

Tumour-intrinsic interferon-γ signalling

A productive T cell response against a tumour antigen results in the expression of interferon-γ (IFNγ) in the tumour microenvironment, which activates Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling, which induces PD-L1 expression. A disruption in tumour cell responses to IFNγ signalling can prevent the induction of PD-L1 expression and thereby render PD1–PD-L1 blockade ineffective (FIG. 1). However, it has long been known that disruption of tumour cell responses to IFNγ signalling is a resistance mechanism not only to immune checkpoint blockade but also more broadly to antitumour immunity. Mouse tumours engineered to express a dominant-negative IFNγ receptor exhibited greater tumorigenicity and were resistant to antitumour immunity elicited by systemic administration of lipopolysaccharide. These tumours could also be established in mice with prior immunity to the parental tumours[11]. When spontaneous tumours arising in mice lacking IFNγ receptor were reimplanted in immunocompetent and immunodeficient mice, they grew with similar kinetics. However, reconstitution of the IFNγ receptor in these tumours caused their rejection in immunocompetent (but not immunodeficient) mice, highlighting the critical role of the tumour-intrinsic IFNγ signalling pathway in immunological rejection[12] (FIG. 2).

Genes encoding proteins of relevance to the IFNγ signalling pathway were also identified in three CRISPR screens designed to identify the genes most relevant to immunotherapy resistance. In one study, guide RNAs targeting 2,368 genes were introduced into a mouse melanoma cell line (B16) engineered to express Cas9 (REF.[13]). These tumours were implanted in wild-type mice that were subsequently treated with anti-PD1 antibodies and GVAX (a vaccine consisting of irradiated tumour cells engineered to overexpress granulocyte–macrophage colony-stimulating factor (GM-CSF)) or were implanted into mice

lacking the T cell receptor (TCR) α-chain locus. Genes that encode proteins involved in the IFNγ receptor signalling pathway, including *Jak1, Stat1, Ifngr1, Ifngr2* and *Jak2*, were highly enriched in tumours arising in wild-type mice treated with anti-PD1 and GVAX. Notably, the study authors identified *Ptpn2*, which encodes a protein that dampens sensitivity to IFNγ receptor signalling, as a mediator of resistance to anti-PD1 and GVAX[13]. In a parallel study, a CRISPR screen was performed on a human melanoma cell line in an in vitro co-culture system with T cells engineered to express a tumour antigen-specific TCR[14]. Again, IFNγ signalling was noted among the pathways that were highly active in resistant tumour cells, with high transcription of genes such as *JAK1* and *STAT1*, along with *APLNR*, which encodes a newly identified regulator of interferon signalling known as apelin receptor. APLNR increased the sensitivity of tumour cells to IFNγ by interacting with JAK1. In a third CRISPR screen, mouse melanoma cells were co-cultured for 3 days with tumour-specific T cells[15]. Yet again, transcripts encoding proteins involved in IFNγ receptor signalling were highly enriched in the resistant tumours compared with tumour cells co-cultured with nonspecific T cells; *Ptpn2* was also enriched in this screen. One caveat to this finding is that the study authors used B16 mouse melanoma cells pretreated with IFNγ to upregulate MHC class I expression, which may bias the results towards the importance of IFNγ signalling. In addition to IFNγ receptor signalling, the study authors found that components of the chromatin regulator PBAF (a form of the SWI/SNF chromatin remodelling complex containing the unique subunits ARID2, PBRM1 and BRD7) suppress the expression of IFNγ response genes and thereby promote resistance of tumour cells to T cell-mediated killing. Genetic deletion of these components from B16 mouse melanoma cells resulted in improved antitumour efficacy of combined anti-PD1 and anti-CTLA4 immune checkpoint blockade in vivo.

These studies confirmed the biological importance of loss-of-function mutations in *JAK1* and *JAK2* seen in patients with melanoma who developed late relapses after successful anti-PD1 therapy[16]. In this scenario, the loss of adaptive PD-L1 expression on tumour cells, while perhaps obviating the need for anti-PD1 therapy, does not explain the acquired resistance to antitumour immunity. Rather, the loss of IFNγ receptor signalling allows the tumour to evade the antitumour effector functions of the immune system. A similar pattern of mutations in IFNγ-related genes has been observed in patients without a response to anti-CTLA4 immune checkpoint blockade[17,18].

In contrast to the role of IFNγ receptor signalling in modulating the immunogenicity of tumours, it has also been proposed that long-term IFNγ receptor signalling in tumour cells can mediate resistance to immune checkpoint blockade. This is based on concepts from antiviral immunity, in which prolonged exposure to type I interferon signalling has deleterious effects on viral control[19,20]. In one study, prolonged exposure of mouse melanoma cells to IFNγ either in vitro or in vivo was shown to result in PD-L1-independent mechanisms of adaptive resistance to immune checkpoint blockade, through upregulation of alternative T cell inhibitory receptors, which was associated with epigenetic and transcriptomic changes related to IFNγ signalling, particularly STAT1 (REF.[21]). Clinical studies combining JAK inhibitors with anti-PD1 checkpoint blockade are ongoing (NCT02646748 and NCT03012230) but early results have not been favourable[22].

It is not entirely clear which of the downstream functions of IFNγ signalling is most critical to the success of immune checkpoint blockade. IFNγ signalling has direct antiproliferative effects[23,24], results in the coordinated expression of antigen processing machinery and surface MHC class I and class II molecules[25,26] and results in the expression of chemoattractants such as CXCL9 and CXCL10 (REFS[27–29]) (FIGS 2,3a). Cell lines derived from human melanoma with intrinsic genetic defects in IFNγ signalling were no longer sensitive to its antiproliferative effects nor did they upregulate MHC class I molecules[18].

Tumour-intrinsic loss of MHC

Tumour cells can evade killing by T cells by downregulating surface MHC expression. Since tumour antigen presentation occurs mainly through the MHC class I pathway, defects in this pathway are more frequently observed than defects in MHC class II antigen presentation. Nevertheless, it has been proposed that MHC class II expression on melanoma cells may be a biomarker of response to anti-PD1 therapy and may be governed by a unique set of resistance mechanisms[30–32].

Much of the importance of IFNγ signalling in antitumour immunity may be related to the fact that it induces or enhances MHC class I antigen presentation, a process that requires coordinated expression of several genes, including *TAP1, TAP2, B2M* and the immunoproteasome genes *PSMB8, PSMB9* and *PSMB10* (FIG. 3b). Tumour cells lacking sensitivity to interferon signalling may have little or no MHC class I antigen presentation, permitting immune escape. In a study from 2001, stable transfection of *TAP1* into tumour cells deficient in IFNγ resulted in their rejection in wild-type but not T cell-deficient (*Rag2*$^{-/-}$) mice[33] (FIG. 2). Indeed, some MHC class I-deficient tumour cells require pretreatment with IFNγ to coordinately express antigen processing machinery and the peptide–MHC class I complex[34].

Defects in the antigen processing machinery disrupt MHC class I surface expression even in the presence of IFNγ signalling[35] (FIGS 2,3b). Not only are tumours with such mutations resistant to T cell-mediated immunotherapy approaches, these mutations may in fact be a result of selective pressure of the immune system. For example, it was reported that patients with melanoma who receive immunotherapy can lose functional expression of β_2-microglobulin (B2M; and thereby MHC class I expression)[36] (FIG. 2). Longitudinal biopsy specimens from another patient with metastatic melanoma demonstrated acquired MHC class I deficiency through loss of *B2M* in the absence of immunotherapy[37]. A recent computational approach to quantify HLA copy number allowed investigators to infer the degree of clonal and subclonal loss of heterozygosity at the HLA locus. Frequent parallel, subclonal and focal HLA loss of heterozygosity events, which are enriched at metastatic sites, suggests an immunological pressure in these tumours even without immunotherapy[38]. A similar association between immunological pressure and genetic alterations in the antigen processing machinery was observed in patients with microsatellite-unstable colorectal cancer, which is highly immunogenic[39].

Unsurprisingly, several cases of acquired resistance to immune checkpoint blockade have been reported with mutations in genes encoding antigen processing machinery, particularly

B2M[16,40]. Moreover, loss of heterozygosity at the *B2M* locus was associated with lower overall survival in two independent cohorts of patients with melanoma treated with immune checkpoint blockade[40]. Novel genes that regulate antigen presentation have also been identified. For example, an in vitro gain-of-function kinome screen showed that *MEX3B*, which encodes a post-transcriptional negative regulator of HLA-A, allows melanoma cells to evade tumour-specific T cells (FIG. 3b). Notably, *MEX3B* expression was enriched a cohort of patients without response after anti-PD1 therapy[41].

Regulation by oncogenic signalling

Oncogenic signalling pathways are likely relevant to tumour immunity across all stages of cancer development, including tumour initiation, growth, invasion and metastasis. The roles of these tumour-intrinsic pathways in shaping tumour immunogenicity and the immune microenvironment were recently reviewed elsewhere[42,43]. Here we focus on three pathways with evidence supporting a role in tumour-intrinsic resistance to immune checkpoint blockade — the WNT–β-catenin pathway, the cyclin-dependent kinase 4 (CDK4)–CDK6 pathway and the mitogen-activated protein kinase (MAPK) pathway — as well as the pathways induced by loss of PTEN.

WNT–β-catenin signalling.—WNT–β-catenin signalling is an evolutionarily conserved signalling pathway involved in a broad range of cell processes, including oncogenesis and embryogenesis. Canonical WNT–β-catenin signalling is initiated by the binding of a WNT family protein to cell surface receptors that activates signal transduction, resulting in nuclear translocation of β-catenin and transcriptional activation. It has recently emerged as an oncogenic signalling pathway that impedes the initiation of de novo antitumour immune responses. This line of reasoning emerged from an observation that the approximately one third of melanoma specimens with active WNT–β-catenin signalling lack significant T cell infiltration. Melanoma cell lines with active WNT–β-catenin signalling produce immunosuppressive cytokines such as IL-10 (REF.[44]). More recently, WNT–β-catenin signalling by melanoma cells in vivo was shown to prevent the priming of antitumour responses by disrupting the recruitment of dendritic cells expressing basic leucine zipper transcriptional factor ATF-like 3 (BATF3)[45,46]. Others have shown that the soluble WNT agonist WNT5A, derived from melanoma cells, can activate β-catenin signalling in dendritic cells, which results in metabolic shifts towards oxidative phosphorylation and fatty acid oxidation, marked by activity of indoleamine 2,3-dioxygenase 1 (IDO1) and peroxisome proliferator-activated receptor-γ (PPARγ), respectively, that promote immunosuppression. Specifically, the conversion of trypto-phan into kynurenine is catalysed by IDO1, which is a transcriptional target downstream of WNT5A-induced signalling[47]. This metabolic shift promotes the development of regulatory T cells while suppressing effector T cell activity. Inhibition of this metabolic shift augments the efficacy of anti-PD1 immunotherapy in a model of *Braf*^{V600E}/*Pten*$^{-/-}$ mouse melanoma[48].

A series of studies across several different types of cancer have indicated a connection between augmented WNT–β-catenin signalling and tumours lacking an intrinsic immune cell infiltrate that are less likely to respond to immune checkpoint blockade (also termed immunologically cold tumours). This includes one study that integrated genomic,

transcriptomic and immunohistochemical data from colorectal cancers in The Cancer Genome Atlas[39], as well as other studies in immunologically cold ovarian cancer, head and neck cancer, bladder cancer and adenoid cystic carcinoma[49–52]. Another study identified serine/threonine-protein kinase PAK4, a WNT signalling mediator, to be enriched in immunologically cold tumours from patients with melanoma not responsive to anti-PD1 immune checkpoint blockade. In multiple mouse models, genetic deletion or pharmacological inhibition of PAK4 resulted in reversal of resistance to anti-PD1 therapy[53].

CDK4–CDK6 and the cell cycle.—Early evidence of a link between cell cycle regulation and oncogenic transformation was obtained from observations of viral transformation coinciding with viral integration at the cyclin A locus, the association of adenoviral oncogene E1A with cyclin A and the overexpression of D-type cyclins in parathyroid tumours[54]. CDK4 and CDK6 are particularly relevant to oncogenesis because, together with D-type cyclins, they promote progression of the cell cycle from G1 phase to S phase. One decade after their discovery, the small molecule palbociclib emerged as the first CDK4/CDK6 inhibitor to gain approval by the US Food and Drug Administration. Since 2017, at least four studies have highlighted the impact of CDK4/CDK6 inhibition on antitumour immunity[55–58]. For example, it was shown that the CDK4/CDK6 inhibitor abemaciclib, in combination with anti-PD-L1 therapy, had a greater antitumour effect in mouse breast cancer models than either agent alone[56]. This observation was attributed to an increased production and sensing of double-stranded RNA (dsRNA) molecules by tumour cells, likely as a result of reduced DNA methyltransferase levels in response to the drug. Tumour cells recognize danger signals such as dsRNA through the expression of pattern recognition receptors, which results in overexpression of proinflammatory genes, including genes encoding interferons and antigen presentation machinery. In another study of human T cells, patient-derived ex vivo cultures and a combination of spontaneous and xenograft mouse models of cancer, the combination of palbociclib or trilaciclib and anti-PD1 blockade was more potent than either agent alone. Here the effects of CDK4/CDK6 inhibition on antitumour immunity were largely attributed to their direct impact on T cells, resulting in greater IL-2 production and increased tumour infiltration, despite lowering their proliferative capacity[58]. Given the role of CDK4/CDK6 in T cell function, whether the effects of CDK4/CDK6 inhibition on antitumour immunity are influenced by a direct effect on oncogenic signalling of tumour cells may depend on the relevance of CDK4/CDK6 in each model system.

A single-cell transcriptomic study of melanoma samples from patients treated with immune checkpoint blockade identified a resistance programme driven by CDK4/CDK6 (REF.[57]). With bulk RNA sequencing data from The Cancer Genome Atlas melanoma cohort, a gene expression signature for tumour cells associated with T cell exclusion was identified, which overlapped with genes enriched in tumours resistant to immune checkpoint blockade. The study authors termed this overlapping gene set the resistance programme. A pharmacological screen of cell lines expressing the resistance programme found these to be sensitive to CDK4/CDK6 inhibitors. Furthermore, in previously published data sets used to study the effect of CDK4/CDK6 inhibition on breast cancer cells and mouse models, the resistance programme was repressed in response to CDK4/CDK6 inhibition. CDK4/CDK6

acts by phosphorylating the tumour suppressor retinoblastoma-associated protein 1 (RB1), and consistent with this, CDK4/CDK6 inhibition repressed the resistance programme in two RB-sufficient melanoma cell lines but not in an RB-insufficient melanoma cell line[57].

MAPK signalling.—The MAPK signalling pathway can play a role in cancer immune evasion by augmenting the expression of the immunoregulatory cytokines IL-6 and IL-10 (REF.[59]). The impact of this signalling pathway on a tumour's immunological status is particularly relevant in melanoma, where approximately half of tumours carry a mutation in the MAPK BRAF, the BRAF-V600E activating mutation, and where immune checkpoint blockade is a first-line therapy. Vemurafenib, an inhibitor of mutated BRAF, was shown to increase the susceptibility of melanoma cells to the cytotoxic effect of T cells, without affecting the proliferative capacity of T cells[60]. This was attributed to the higher expression of MHC class I molecules and melanoma differentiation antigens[61,62].

BRAF-V600E

A specific activating mutation in the *BRAF* gene commonly found in human melanoma, which results in increased cell growth.

Vemurafenib can also induce cell cycle arrest through cooperative signalling through the IFNγ receptor and the tumour necrosis factor receptor in a manner dependent on activating mutation $BRAF^{V600E}$ (REF.[63]). In support of this finding, the CRISPR screen of B16 tumour cells in co-culture with tumour-specific T cells described earlier also showed that resistant tumour cells were enriched for CRISPR guides targeting negative regulators of the MAPK pathway[15]. In a separate study using RNA sequencing of bulk melanoma specimens from patients treated with PD1 blockade, a gene signature was identified for tumour samples taken from patients who did not show a response, which overlapped with a previously published signature associated with resistance to MAPK inhibitors[64]. However, these data must be evaluated cautiously, as they were not corroborated by two other large transcriptomic data sets derived from tumours of patients with melanoma treated with immune checkpoint blockade[57,65]. BRAF inhibition can also disrupt tumour-intrinsic expression of immunosuppressive factors. For example, $BRAF^{V600E}$ tumours show increased expression of the cytokines IL-6, VEGF and IL-10, which have immunosuppressive functions in part through their effect on dendritic cell function (such as IL-12 and tumour necrosis factor production)[59].

The development of combination therapies using immune checkpoint blockade and inhibition of mutant BRAF with vemurafenib stalled due to toxicity concerns related to paradoxical activation of the MAPK pathway in wild-type BRAF cells. Instead, investigators turned to the use of MAPK/extracellular signal-regulated kinase kinase (MEK) inhibitors, which inhibit the MAPK pathway in both BRAF-V600E and wild-type BRAF cells, or combinations of MEK and BRAF inhibitors. In a preclinical model of colon cancer (the CT26 model), anti-PD1 therapy in combination with MEK inhibition resulted in long-lasting tumour control[66]. Similarly, the combination of MEK and BRAF inhibition augmented the efficacy of both adoptive T cell therapy and anti-PD1 blockade[67]. Three clinical studies combining inhibitors of MAPK signalling and immune checkpoint blockade were recently

reported[68–70]. Two of these studies used a combination of dabrafenib (a BRAF inhibitor), trametinib (a MEK inhibitor) and the anti-PD1 antibody pembrolizumab, and both observed high response rates (63% and 73%), coupled with high rates of grade 3 or higher toxic effects (58% and 73%, respectively). The third study included a cohort of patients receiving a combination of cobimetinib (a MEK inhibitor), vemurafenib and the anti-PD-L1 antibody atezolizumab, of whom 72% achieved objective responses (complete response rate of 21%). A lead-in period of cobimetinib and vemurafenib therapy resulted in a relative increase in the levels of circulating proliferative $CD4^+$ T cells[68–70].

Loss of the tumour suppressor PTEN.—Although the canonical role of tumour suppressors in oncogenesis has been described for a century, the discovery of *PTEN* loss as a common oncogenic event was not identified until the end of the twentieth century[71,72]. One study rigorously examined the role of *PTEN* deletion in both human melanoma and a syngeneic mouse model of melanoma with respect to the efficacy of T cell-based immunotherapy[73]. In the absence of *PTEN*, tumour cells were more resistant to the cytotoxic effects of tumour-specific T cells both in vitro and in vivo. *PTEN* expression also correlated with response to anti-PD1 therapy and a more successful yield of ex vivo expanded tumour-infiltrating lymphocytes from patients. Other studies have observed similar effects of *PTEN* loss: RNA sequencing data from The Cancer Genome Atlas soft tissue sarcoma data set reveals decreased expression of genes associated with T cell infiltration and cytolytic activity (such as the genes encoding CD8α and granzyme B) in tumours with deletion of *PTEN*. Furthermore, in a patient with a partial response to anti-PD1 immune checkpoint blockade, a non-responding lesion was found to have a deletion of *PTEN*[74], suggesting a possible role for *PTEN* deletion in resistance to therapy. Notably, PTEN can promote type I interferon signalling in response to viral stimuli by aiding in the nuclear translocation of interferon regulatory factor 3 (IRF3) in response to the activation of pattern recognition receptors by DNA viruses, RNA viruses, polyinosinic:polycytidylic acid and lipoolysaccharide[75]. This may have future relevance in the use of emerging drugs that target pattern recognition receptors to overcome resistance to immune checkpoint blockade.

Inhibition of phosphoinositide 3-kinase (PI3K) has been proposed as a therapeutic approach to promote antitumour immunity given that PI3K is negatively regulated by PTEN and is a commonly dysregulated kinase in cancer. However, different PI3K isoforms are active in cancer cells (PI3Kα and PI3Kβ) and immune cells (PI3Kδ and PI3Kγ). Although inhibition of isoforms of PI3K that are enriched in tumour cells can reduce tumour growth, most of the evidence supporting a role for PI3K inhibition in improving antitumour immunity is based on inhibition of PI3Kγ or PI3Kδ. For example, wild-type tumours growing in hosts that lack functional PI3Kγ or PI3Kδ have slowed tumour growth in a T cell-dependent manner[76,77]. PI3Kγ activation in macrophages can activate an immunosuppressive transcriptional programme that prevents antitumour T cell function in a tumour cell-independent manner[78]. However, whether tumour-intrinsic PI3K inhibition impacts antitumour immunity is less clear. Clinical studies are ongoing (such as NCT02646748) to assess the combined impact of PI3K inhibitors and immune checkpoint blockade in patients with solid tumours.

Tumour dedifferentiation and stemness.—Tumour-initiating or tumour stem cells are resistant to traditional cytotoxic therapies. Evidence has emerged to suggest that tumour dedifferentiation or stemness may also play a role in resistance to immune-based therapies. Transcriptomic analysis of tumours from patients with melanoma resistant to anti-PD1 immune checkpoint blockade identified an enrichment of a stem-like mesen-chymal gene signature[64]. In one patient who responded to adoptively transferred T cells targeting the melanocyte differentiation antigen 1 (MART1), relapsed tumours lost expression of MART1, a phenomenon of dedifferentiation and immunotherapy resistance that was phenocopied in vitro[79]. Other studies have demonstrated that tumour-initiating stem cells may express negative regulatory molecules, such as CD80 (REF.[80]), PD-L1 (REF.[81]) and NKGD2 (REF.[82]). Lastly, WNT signalling, described earlier as an oncogenic pathway mediating immunotherapy resistance, also has a well-described role in tumour stemness and dedifferentiation[83].

Biomarkers of tumour-intrinsic resistance

De novo tumour-reactive T cells

Unlike approaches based on adoptive transfer of activated tumour-specific T cells, immune checkpoint blockade harnesses naturally occurring antitumour T cell responses. Several observations imply that the efficacy of immune checkpoint blockade depends on a pre-existing immune response. Firstly, patients with melanoma, a tumour known for its inherent immunogenicity, have a high response rate to immune checkpoint blockade monotherapy[84]. Melanoma has long been a model malignancy for studying immunotherapy, and the clinical success of adoptive cell transfer of ex vivo expanded tumour-infiltrating lymphocytes in patients with metastatic melanoma provides evidence for the presence of naturally occurring tumour-specific T cells in patients with melanoma[85].

The simplest indicator of a pre-existing antitumour immune response may be the presence of T cells within the tumour microenvironment[86–89]. Among patients with melanoma receiving anti-PD1 therapy, the presence of T cells in pretreatment biopsy specimens was associated with response to therapy[90,91] and $CD8^+$ T cell density at the invasive margin was predictive of response in a small validation cohort[91]. Baseline specimens from patients responding to PD1 blockade therapy also had higher levels of phosphorylated STAT1 expression at the invasive margin. This suggests that a response to therapy requires not only the presence of T cells but also the presence of activated T cells producing IFNγ, which initiates a signalling cascade that leads to the phosphorylation of STAT1 in the adjacent tumour and stromal cells. This supports the role of tumour-intrinsic IFNγ signalling in the response to PD1 blockade described above. Similar findings were reported in a study of anti-PD1 therapy (pembrolizumab) in patients with colorectal cancer with mismatch repair deficiency[92], and anti-PD-L1 therapy in patients with urothelial carcinoma[93].

However, this observation has not been universal; tumour-infiltrating lymphocytes present at the baseline were not associated with response to anti-PD1 therapy in a cohort of patients who previously had either received anti-CTLA4 therapy or not received it[65], and there are exceptions in other cohorts as well. This may in part be related to tumour heterogeneity and selection bias of pretreatment biopsy specimens used for analysis. There

may also be a subset of cases in which patients harbour tumour-specific T cells but local immunosuppressive factors limit the infiltration and expansion of these clones. In other cases, tumour-specific T cells may be present in the periphery but not in the tumour microenvironment[94]. This indicates that the presence of tumour-infiltrating lymphocytes is not a particularly sensitive surrogate for the presence of a de novo antitumour immune response.

PD-L1 as a marker of interferon signalling

The expression of immune checkpoint molecules, such as PD-L1, within the tumour microenvironment has been shown to predict response to immune checkpoint blockade in some but not all cases[95,96]. However, PD-L1 expression does not necessarily indicate a pre-existing antitumour immune response. Some patients with PD-L1-positive tumours fail to respond to therapy, and some patients with PD-L1-negative tumours can also derive a benefit from immune checkpoint blockade[97–99].

The expression of PD-L1 is primarily regulated by the interferon signalling pathway, which includes the kinases JAK1 and JAK2, as well as the transcription factors STAT1, STAT2 and STAT3, and the transcriptional activator IRF1 (REFS[100,101]). IFNγ can even stimulate the expression of PD-L1 on tumour-derived exosomes, which can also mediate suppression of $CD8^+$ T cells[102]. In this scenario, in which tumour-infiltrating T cells coexist with PD-L1-expressing tumour and/or immune cells, blockade of the PD1–PD-L1 axis is likely to be effective (FIG. 1a), and further supports the role of tumour-intrinsic IFNγ signalling in the response to PD1 blockade.

Both type I interferon signalling and type II interferon (IFNγ) signalling converge to activate similar downstream gene targets such as *PDL1*. Whereas type I interferons are primarily produced by myeloid cells in response to activation of pattern recognition receptors, type II interferon is primarily produced by T cells on recognition of cognate antigen. Thus, in the context of T cell-based antitumour immunity, type II interferon plays a more prominent role. Mutations in the interferon signalling pathways (especially, type II interferon signalling), or epigenetic and post-transcriptional mechanisms that limit tumour-specific PD-L1 expression, can render PD-1–PD-L1 immune checkpoint blockade redundant[13,103] (FIG. 1b).

PD-L1 expression can also be modulated through various other mechanisms. These include genetic overexpression (such as amplification of the loci for PD-L1, PD-L2 and JAK2, known as the PDJ amplicon[104]), epigenetic silencing, transcriptional regulation (for example, by MYC, PTEN and hypoxia-inducible factor 1α), post-transcriptional regulation (by micro-RNAs), post-translational modifications (glycosylation, phosphorylation and ubiquitylation) and cytoplasmic and endosomal relocation. These processes, which can also impact response to PD1 blockade therapy, are reviewed in detail elsewhere[105].

Lessons from the tumour transcriptome

Immunohistochemistry-based methods to assess the immunological status of tumours have been limited by the dimensionality of their analysis, emerging multiplex approaches notwithstanding. As such, they have been outpaced by RNA sequencing and targeted gene arrays. These efforts, in parallel with advances in RNA deconvolution algorithms such as the

cytolytic activity score, MCP-counter, CIBERSORT and TIMER[106–110], allow assessments of the immune cell composition of a bulk tumour specimen. The cytolytic activity score is the simplest of the RNA deconvolution techniques and summarizes effector T cell composition of a tumour using the geometric mean of the expression of granzyme A and perforin[111]. Higher baseline cytolytic activity scores correlate with response to anti-CTLA4 immune checkpoint blockade[112], as does a viral defence gene expression signature[113].

Bulk tumour RNA-based immune signatures, though, have drawbacks. Tumour heterogeneity is a hurdle to reproducible, consistent results both within and across studies. In a cohort of patients pretreated with anti-CTLA4 (but not anti-CTLA4 treatment-naive patients), the cytolytic activity score was increased and viral defence signatures were enriched in baseline tumours of anti-PD1-responsive patients, but no specific immune populations identified by CIBERSORT RNA deconvolution at baseline were significantly associated with response[65]. In a separate cohort of patients treated with anti-PD1, baseline cytolytic activity score or interferon signatures were not associated with response[64].

To overcome the hurdles of bulk tumour transcriptomics, single-cell RNA sequencing efforts are ongoing. An analysis of 48 tumour biopsy specimens from 32 patients treated with immune checkpoint blockade showed that $CD8^+$ T cell infiltration (defined by immunohistochemistry) was not increased in baseline specimens from responding patients[114]. However, single-cell RNA sequencing revealed that the $CD8^+$ T cells in baseline specimens of responders were enriched in transcripts related to memory cell differentiation (for example, *TCF7*, which encodes a transcription factor), activation and cell survival compared with $CD8^+$ T cells in non-responders, which were enriched in genes related to exhaustion[114].

Tumour neoantigens as T cell targets

Despite the evidence that neoantigen-specific T cell responses are central to the efficacy of immune checkpoint blockade, mutational burden is limited as a predictor of response to immune checkpoint blockade[115]. This may be partly due to the clonality of the mutations in question. Clonal mutations, which are shared across all tumour cells in a patient, may be more critical to the generation of an effective antitumour response[116].

Furthermore, for a mutation to serve as an immunological target, it must be effectively presented to the immune system by MHC antigens. Although neoantigen prediction tools have improved, the lack of high-throughput assays to validate these prediction tools has limited their progress[117,118]. For example, in a cohort of patients who received anti-CTLA4 therapy, the predicted neoantigen burden did not outperform mutational burden as a biomarker for response[119]. Lastly, the presence of neoantigens is likely an insufficient biomarker given the presence of other obstacles to an antitumour immune response in the tumour microenvironment[120]. Indeed, a biomarker that captured both tumour-intrinsic mutational burden and an inflamed tumour microenvironment was more strongly associated with response across multiple prospective studies of anti-PD1 therapy than either biomarker alone[121].

Overcoming tumour-intrinsic resistance

The superior antitumour responses seen with the combination of anti-CTLA4 and anti-PD1 blockade compared with either therapy alone indicate a non-redundant molecular mechanism of these two immunological checkpoints (recently reviewed elsewhere[122]). Transcriptomic and immunohistochemical data suggest that responders to dual immune checkpoint blockade are those with pre-existing productive antitumour responses held in check by immune checkpoints beyond the level of PD1–PD-L1 and CTLA4 blockade[65,91,114] (FIG. 4a). Several inhibitors targeting alternative immune checkpoints are in preclinical and clinical stages of development, including those targeting LAG3, VISTA, TIM3, adenosine A2A receptor, CD73, BTLA, B7-H3, B7-H4 and killer cell immunoglobulin-like receptors[122,123].

However, this leaves a large fraction of patients who have immunologically cold tumours and are unlikely to respond to single or combination immune checkpoint blockade. For these patients, the aim is to initiate antitumour immune responses by enhancing antigen presentation and priming immune responses against existing antigens. The tumour and its draining lymph nodes have been purported as the predominant site of tumour antigen presentation[124], and thus tumour-directed approaches to modulate intratumoural and lymph node antigen presentation are of interest (FIG. 4b). These approaches are based on (1) inducing a proinflammatory state that overwhelms the basal mechanisms of immunosuppression in the tumour microenvironment, (2) inducing immunogenic cell death and (3) recruiting professional antigen-presenting cells (APCs) for efficient priming against tumour antigens. One early example of an intratumoural immune stimulant is bacillus Calmette–Guérin, which is a standard therapy for superficial bladder cancer[125].

Chemotherapy and radiation therapy can both induce immunogenic cell death through a variety of proposed mechanisms, which are reviewed in detail elsewhere[126–130]. In mouse models, it was shown that the immune effects of both chemotherapy and radiation therapy are dependent on T cells and both can augment the impact of immune checkpoint blockade[131–133]. However, chemotherapy and radiation therapy have well-documented immunosuppressive functions that induce tumour-extrinsic mechanisms of resistance to immunotherapy[134]. Therefore, it is unlikely that these standard therapies will emerge as a primary approach to overcome intrinsic resistance to immune checkpoint blockade, but their role in controlling disease burden and eliciting immunogenic cell death may prove useful in combination with emerging combination immunotherapies.

Several immunotherapeutic strategies target pattern recognition receptors, using oncolytic viruses or viral mimicry. Viral mimicry can be accomplished using compounds such as polyinosinic:polycytidylic acid, which mimics dsRNA and activates TLR3, MDA5 and RIG-I, or CpG oligodeoxynucleotides, which mimic single-stranded DNA and activate TLR9. Activation of pattern recognition receptors results in downstream activation of proinflammatory genes including genes that encode type I interferons, which can start a cascade that recruits and activates APCs (such as dendritic cells) that are critical for the initiation of an antitumour immune response[135].

Oncolytic viruses have a unique capacity to infect tumour cells and induce cell death; for therapeutic purposes they are also often genetically engineered to potentiate antitumour immune responses. Talimogene laherparepvec (or T-VEC, marketed as Imlygic), which is based on a herpes simplex type 1 virus, was the first oncolytic virus to gain US Food and Drug Administration approval. It is delivered intratumourally in patients with metastatic melanoma, where it preferentially replicates within tumour cells and expresses the cytokine GM-CSF to promote the maturation and activation of APCs in the vicinity[136]. T-VEC is engineered not to interfere with antigen presentation in infected cells, unlike the viral vector it is derived from. In combination with anti-PD1 therapy, T-VEC resulted in an objective response rate of 62% in a phase Ib study[137] in patients with metastatic melanoma, a greater response rate than would be expected with anti-PD1 therapy alone. Most notably, 9 of 13 patients with low $CD8^+$ T cell infiltration had objective responses and 3 of 5 patients with low baseline IFNγ production had complete responses, supporting a role for T-VEC in patients without pre-existing antitumour immune responses. The proposed mechanism is as follows: while virus-mediated immunogenic cell death results in the availability of peptide antigens, innate sensors of viral antigens promote IFNγ signalling. This, together with the enforced expression of GM–CSF by T-VEC, results in the recruitment and activation of APCs in the tumour microenvironment. APCs then either prime or activate tumour-specific T cells in the tumour microenvironment or draining lymph nodes, reversing the pre-existing immune exclusion established by the tumour.

Non-virus-based tumour-directed approaches to enhance tumour immunogenicity include activators of pattern recognition receptors such as SD-101, a synthetic oligonucleotide with CpG motifs that activates TLR9 signalling on both tumour and non-tumour cells within the tumour microenvironment. In a phase Ib study, 78% of patients with melanoma who had not previously been treated with anti-PD-1 had an objective response[138]. Preclinical studies in multiple mouse models supported the use of TLR9 agonist CpG oligonucleotides to induce systemic antitumour immunity[139,140]. These included a combination of SD-101 and an OX40 agonist antibody that was effective in multiple models, including a spontaneous mouse model of metastatic breast cancer[141]. It is also plausible that agonists of innate immune sensors, which are potent inducers of type I interferon signalling, can provide stimuli for antigen presentation in tumours that are resistant to immune checkpoint blockade owing to genetic or epigenetic deficiencies in type II interferon signalling (FIG. 4c).

The sensitivity of tumour cells to immune checkpoint blockade is fine-tuned by their intrinsic sensitivity to endogenous innate immune signals, such as endogenous dsRNA. Altering the set point of tumour cells to endogenous dsRNA (that is, reducing the dsRNA threshold) may be an avenue to overcome tumour-intrinsic resistance to immune checkpoint blockade. In an in vivo CRISPR screen targeting more than 2,300 genes in mouse melanoma cells, the loss of *ADAR1*, which encodes an RNA-editing enzyme that converts adenosine into inosine, was increased in tumours with better response to anti-PD1 and GVAX[13]; loss of *ADAR1* in B16 mouse melanoma reversed the immunologically cold state of the tumour microenvironment and increased the sensitivity of tumour cells to the direct antitumour effects of type I or type II interferons[142]. The improved response to anti-PD1 therapy in ADAR1-deficient tumours was dependent on the presence of at least one of the two dsRNA sensors MDA5 and PKR. These data support the role of tumour-intrinsic RNA sensing

in the efficacy of immune checkpoint blockade. The role of tumour-intrinsic sensing of cytoplasmic DNA, however, is still under investigation.

Host innate immune sensing plays a well-described role in immune checkpoint responsiveness[143] but may be dispensable under the right conditions. Pancreatic ductal adenocarcinoma has minimal T cell infiltration and a poor response to immune checkpoint blockade[144]. The combination of chemotherapy, an agonist of the costimulatory protein CD40 and anti-PD1 therapy results in T cell-dependent antitumour efficacy in a mouse model of pancreatic cancer[145]. CD40 is expressed broadly across immune cells, including dendritic cells, and its engagement by CD40 ligand is known to license antigen presentation[146]. In combination with chemotherapy and anti-PD1 therapy, CD40 activation and chemotherapy-induced immunogenic cell death drive T cell activation in a $BATF3^{+}$ dendritic cell-dependent manner, but independently of host innate immune signalling pathways, including those that signal through MYD88, TLR4, TRIF, TLR3, STING, P2X74, caspase 1 and caspase 11. A phase I/II study of CD40 agonist in combination with chemotherapy and anti-PD1 therapy is ongoing (NCT03214250).

At least two immune-based approaches are being investigated for patients harbouring tumours with genetic defects that impair MHC class I or class II antigen presentation (FIG. 4d). Chimeric antigen receptor (CAR)-based adoptive T cell therapy is a potent immunotherapy for haematological malignancies that bypasses the need for antigen presentation through MHC as it directly targets specific surface molecules expressed by tumour cells. However, success in the treatment of solid tumours has been elusive for CAR T cells owing to a paucity of tumour-specific surface antigens and an immunosuppressive microenvironment. Novel engineering approaches to create dual-target activated CAR T cells, CAR T cells with synthetic ‘AND-gate’ logic switches to promote safety and target specificity and CAR T cells that are insensitive to or co-opt immunosuppressive signalling within the tumour microenvironment (such as transforming growth factor-β signalling) sustain promise for this approach[147–150]. Another approach against MHC-deficient tumours is the use of cellular therapies using natural killer (NK) cells, which function to eliminate cells lacking MHC class I molecules[151]. B2M-deficient B16 tumours lacking ADAR, which were sensitized to a combination of GVAX and anti-PD1 therapy, were noted to have an increase in NK cell infiltration[142]. Adoptive NK cell therapy approaches have been under investigation for several years[152], and more recently, blockade of NKG2A, a tyrosine-based inhibitory motif expressed on both NK cells and T cells, has demonstrated activity in patients with head and neck squamous cell carcinoma[153].

Synthetic ‘AND-gate’ logic switches

Type of chimeric antigen receptor constructs that use synthetic Notch receptors. sensing of ligand by the synthetic Notch receptor induces transcription of a chimeric antigen receptor that is specific for a second ligand. T cell activation is achieved only when both ligands are present.

Lastly, to address mechanisms of immune checkpoint blockade resistance driven by oncogenic signalling (FIG. 5a), investigators have repurposed existing inhibitors of

oncogenic signalling pathways as an approach to boost antitumour immunity (especially in combination with immune checkpoint blockade). These include inhibitors of WNT signalling, inhibitors of CDK4 and CDK6 and inhibitors of MAPK and PI3K (illustrated in FIG. 5b).

Conclusion

The process of identifying mechanisms of resistance to immune checkpoint blockade has been a rediscovery of the central mechanisms regulating antitumour immunity. Tumour sensitivity to immune checkpoint blockade is dictated by tumour biology: patients with tumours that have shared histological, molecular and genetic features have similar response rates to immune checkpoint blockade. Tumour-intrinsic factors — through their effect on the interplay between the tumour and the host immune system — can indirectly play a role in tumour-extrinsic mechanisms of resistance. However, we focused this Review on resistance mechanisms directly impacted by tumour-intrinsic factors. Tumours that activate a de novo antitumour immune response, as a result of increased mutational burden and antigenicity, are most likely to benefit from immune checkpoint blockade. However, even with sufficient antigenicity, sensitivity to immune checkpoint blockade can be disrupted by tumour-intrinsic genetic defects in IFNγ signalling and antigen presentation. Oncogenic signalling pathways, by dictating the recruitment of cells critical for initiating and effecting the antitumour immune response, impacting IFNγ and antigen presentation pathways, or by inducing immunosuppressive factors in the tumour microenvironment, are also mediators of immune checkpoint blockade resistance. Targeted approaches to bypass defects in IFNγ signalling and antigen presentation or to inhibit immunosuppressive oncogenic signalling pathways hold promise in broadening the impact of immune checkpoint blockade.

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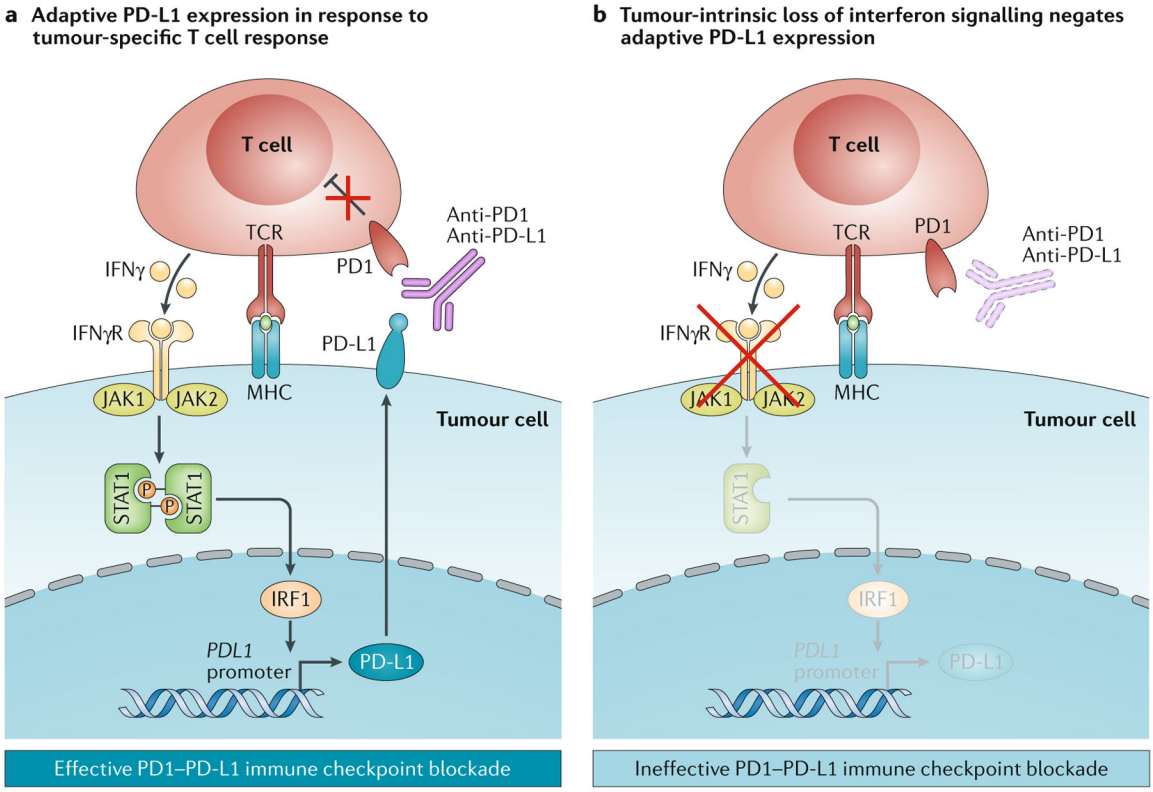

Fig. 1 |. Interferon signalling in adaptive programmed cell death 1 ligand 1 expression.

a | A pre-existing antitumour immune response is essential for effective immune checkpoint blockade. Tumour-reactive T cells, which recognize tumour neoantigens in the context of MHC class I or class II, release interferon-γ (IFNγ), resulting in activation of the Janus kinase (JAK)– signal transducer and activator of transcription (STAT) signalling pathway. This activates the transcription factor interferon regulatory factor 1 (IRF1), which then activates the transcription of *PDL1*. This results in adaptive expression of programmed cell death 1 ligand 1 (PD-L1) on the surface of tumour cells, which negatively regulates the antitumour T cell response. Antibodies against PD1 or PD-L1 disrupt this negative feedback loop to restore antitumour immunity. **b** | A similar scenario in which tumour-specific T cells encounter antigen in the context of MHC, resulting in the release of IFNγ. However, here the IFNγ signal is not transmitted by the tumour cell owing to genetic deficiencies in the IFNγ signalling pathway (affecting, for example, JAK1 or JAK2) and adaptive PD-L1 expression does not occur. In the absence of adaptive PD-L1 expression, PD1–PD-L1 immune checkpoint blockade is ineffective. IFNγR, IFNγ receptor; TCR, T cell receptor.

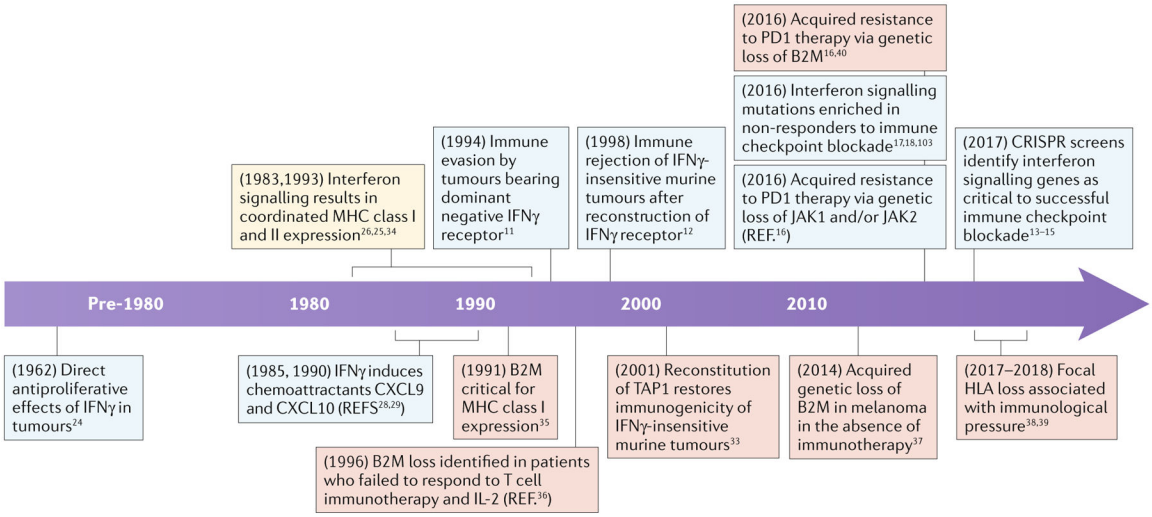

Fig. 2 |. Timeline of original discoveries of the importance of the interferon-γ (IFnγ) pathway and antigen presentation in antitumour immunity.

This timeline also highlights the rediscovery of these key pathways in the era of immune checkpoint blockade after 2011. Discoveries relating to the IFNγ pathway are shown in blue, discoveries relating to antigen presentation are shown in pink and discoveries relating to both the IFNγ pathway and antigen presentation are shown in light pink. B2M, β2-microglobulin; JAK, Janus kinase; PD1, programmed cell death 1.

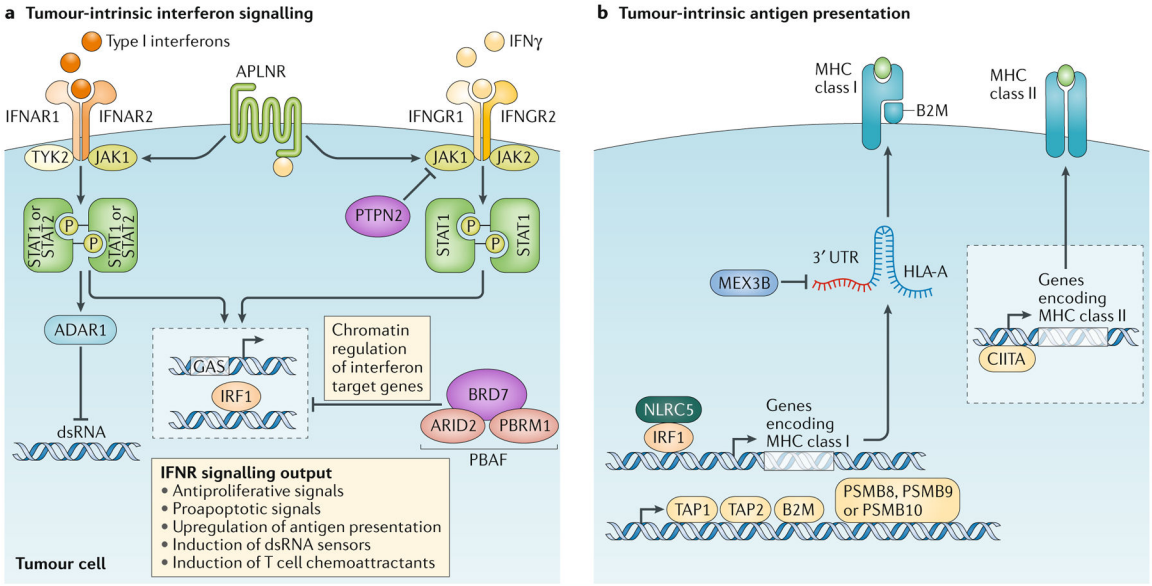

Fig. 3 |. Resistance to immune checkpoint blockade: tumour-intrinsic escape mechanisms.

a | Multiple unbiased CRISPR-based screens have uncovered the critical role of tumour-intrinsic interferon signalling in response to immune checkpoint blockade and T cell-based immunotherapy[13–15]. These studies have identified components of the interferon-γ (IFNγ) and type I interferon signalling pathway such as Janus kinase 1 (JAK1), JAK2, signal transducer and activator of transcription 1 (STAT1) and IFNγ receptor I (IFNGR1) and IFNGR2 as critical to the success or failure of immune checkpoint blockade. The studies also identifed a role in responses to immune checkpoint blockade for lesser known regulators include the surface receptor apelin receptor (APLNR), which modulates upstream sensitivity to IFNγ and type I interferon signalling, tyrosine-protein phosphatase non-receptor type 2 (PTPN2), which modulates upstream sensitivity to IFNγ signalling, BRD7 and the DNA-binding subunits ARID2 and PBRM1, which are part of the chromatin remodelling complex PBAF and are involved in the regulation of IFNγ target genes; and the enzyme double-stranded RNA (dsRNA)-specific adenosine deaminase (ADAR1), which negatively regulates endogenous dsRNA levels. Type I interferon and IFNγ signalling converge on IFNγ activation sites (GAS) in the DNA and activate transcriptional regulators such as interferon regulatory factor 1 (IRF1), which then drive key outputs from interferon signalling. **b** | A number of studies showed that interferon-independent defects in antigen presentation can also lead to immune evasion and resistance to immune checkpoint blockade. These defects can occur in the HLA loci or in the MHC class I complex component β2-microglobulin (B2M)[16,40]. Other defects can occur in the antigen processing machinery, such as the membrane-bound transporter proteins TAP1 and TAP2 and the immunoproteasome subunits PSMB8, PSMB9 or PSMB10, or in the transcriptional regulation of MHC class I (such as the cytoplasmic protein NLRC*5*). MHC class I expression can also be affected at the post-transcriptional level. The RNA-binding protein MEX3B can bind HLA-A transcripts, resulting in their degradation and reduced expression of MHC class I molecules. MEX3B was found to be upregulated in patients with melanoma who did not respond to checkpoint blockade[41]. Although higher MHC class II antigen presentation on tumour cells has been associated with improved responses to

immune checkpoint blockade[31], genetic defects in MHC class II genes or the MHC class II transcriptional activator CIITA have not been identified in cases of resistance to immune checkpoint blockade. IFNAR1, interferon-α and β receptor subunit 1; IFNR, interferon receptor; UTR, untranslated region.

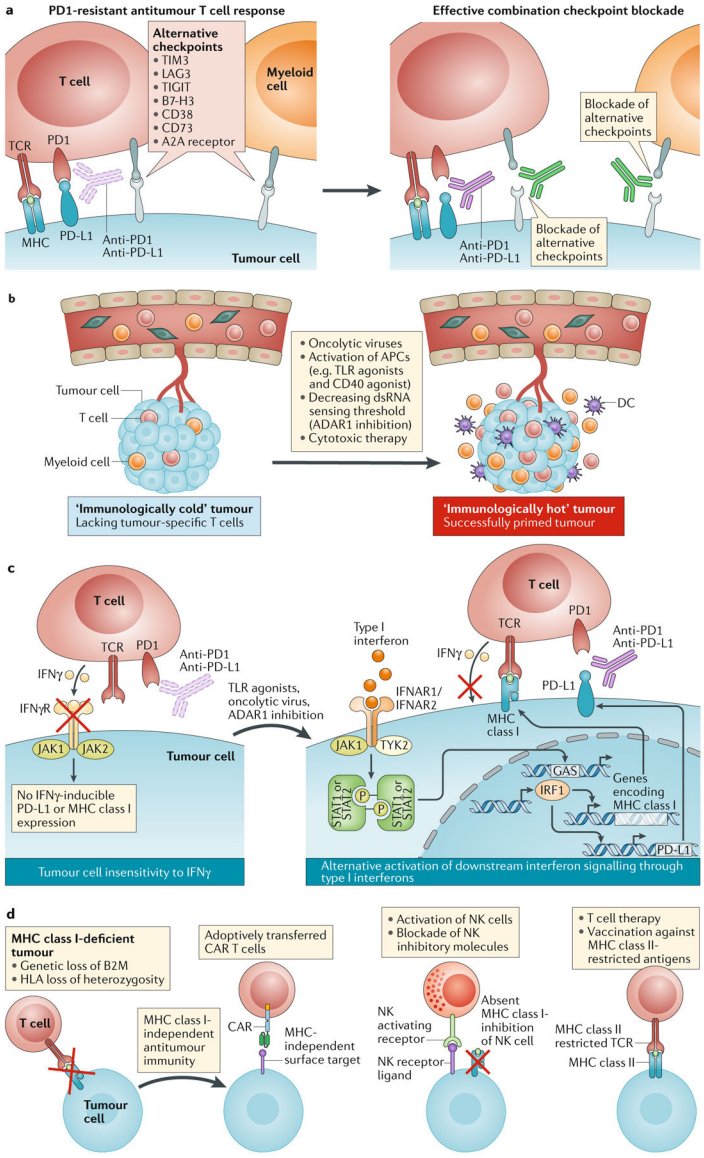

Fig. 4 |. Overcoming tumour-intrinsic resistance to immune checkpoint blockade.

a | The programmed cell death 1 (PD1)–PD1 ligand 1 (PD-L1) axis may not be the sole negative regulator of antitumour T cell responses. Alternative immune checkpoint molecules expressed on tumour cells, or myeloid cells in the tumour microenvironment, prevent effective antitumour immunity; combined immune checkpoint blockade may disrupt this resistance mechanism (right panel). **b** | Immunologically cold tumour types lack pre-existing antitumour T cell responses, rendering immune checkpoint blockade ineffective. Approaches to prime the immune system against tumours by causing immunogenic cell death (oncolytic viruses or cytotoxic therapy), priming antigen-presenting cells (APCs; using Toll-like receptor (TLR) agonists and CD40 agonists) or increasing tumour cell sensitivity to double-stranded RNA (dsRNA; such as inhibition of dsRNA-specific adenosine deaminase (ADAR1)) can reprogramme the immunologically cold state into a checkpoint blockade responsive state. **c** | Tumours without interferon-γ (IFNγ) signalling lack the capacity to

adaptively express PD-L1 in response to IFNγ. In some tumours in which MHC class I antigen presentation is largely dependent on IFNγ signalling, loss of IFNγ signalling equates to loss of antigen presentation. Activation of the alternative interferon pathway (type I interferon) through TLR agonists, oncolytic viruses or other means, can also result in activation of signal transducer and activator of transcription 1 (STAT1) and STAT2 signalling, which drives transcription of PD-L1 and MHC class I via the induction of interferon regulatory factor 1 (IRF1). **d** | Other tumours are resistant to immune checkpoint blockade after loss of MHC class I expression via genetic alterations (such as loss of β2-microglobulin (B2M) and loss of HLA heterozygosity). Three approaches can be successful in this setting: (1) chimeric antigen receptor (CAR) T cells recognize their targets independently of MHC class I expression; (2) adoptive transfer of natural killer (NK) cells or NK cell stimulation with cytokines such as IL-2 or IL-15, as these target cells lack MHC class I expression; and (3) vaccination or adoptive T cell therapy to generate responses against a specific MHC class II-restricted antigen. B2M, β2-microglobulin; DC, dendritic cell; GAS, IFNγ activation sites; IFNAR1, interferon-α and β receptor subunit 1; IFNγR, IFNγ receptor; JAK1, Janus kinase 1; TCR, T cell receptor.

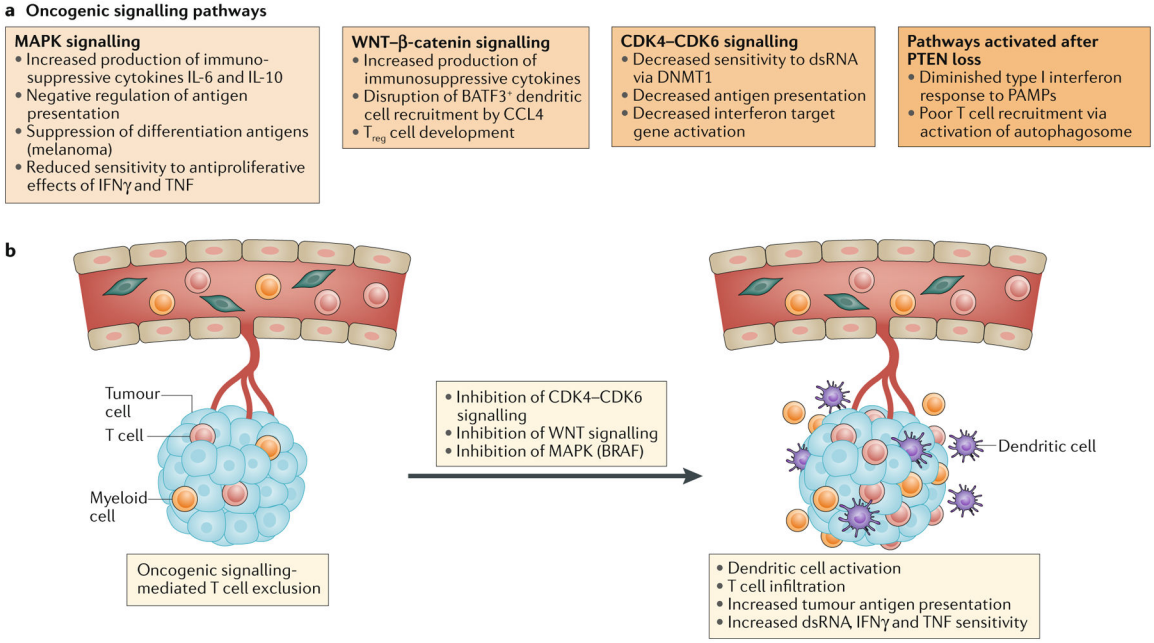

Fig. 5 |. Oncogenic signalling pathways affecting antitumour immunity and resistance to immune checkpoint blockade.

a | Oncogenic signalling pathways provide unique tumour-intrinsic mechanisms of immune evasion. Here we highlight four key oncogenic signalling pathways implicated in antitumour immunity: the mitogen-activated protein kinase (MAPK) signalling pathway, the WNT–β-catenin pathway, the cyclin-dependent kinase 4 (CDK4)–CDK6 cell cycle signalling pathway and pathways activated as a result of loss of the phosphoinositide phosphatase PTEN. **b** | Therapeutic disruption of CDK4–CDK6 signalling (for example with palbociclib or abemaciclib), MAKP signalling (BRAF inhibitors) or WNT signalling can reverse the tumour-intrinsic T cell-excluded state and restore sensitivity to immune checkpoint blockade. BATF3, basic leucine zipper transcriptional factor ATF-like 3; CCL4, CC-chemokine ligand 4; DNMT1, DNA (cytosine-5)-methyltransferase 1; dsRNA, double-stranded RNA; IFNγ, interferon-γ; PAMPs, pathogen-associated molecular patterns; TNF, tumour necrosis factor; T_{reg} cell, regulatory T cell.