Mechanisms of immune activation and regulation

Mechanisms of immune activation and regulation: lessons from melanoma

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Abstract | Melanoma, a skin cancer that develops from pigment cells, has been studied intensively, particularly in terms of the immune response to tumours, and has been used as a model for the development of immunotherapy. This is due, in part, to the high mutational burden observed in melanomas, which increases both their immunogenicity and the infiltration of immune cells into the tumours, compared with other types of cancers. The immune response to melanomas involves a complex set of components and interactions. As the tumour evolves, it accumulates an increasing number of genetic and epigenetic alterations, some of which contribute to the immunogenicity of the tumour cells and the infiltration of immune cells. However, tumour evolution also enables the development of resistance mechanisms, which, in turn, lead to tumour immune escape. Understanding the interactions between melanoma tumour cells and the immune system, and the evolving changes within the melanoma tumour cells, the immune system and the microenvironment, is essential for the development of new cancer therapies. However, current research suggests that other extrinsic factors, such as the microbiome, may play a role in the immune response to melanomas. Here, we review the mechanisms underlying the immune response in the tumour and discuss recent advances as well as strategies for treatment development.

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Cutaneous melanoma is a malignancy of melanocytes for which sun ultraviolet exposure is the major risk factor, leading to a high mutational load. Melanoma tumours display many different driver-gene and passenger-gene mutations associated with tumour cell survival and proliferation[1]. Some of these mutations have been targeted with rationally designed therapies using small-molecule agents. For example, vemurafenib and dabrafenib, approved for use in *BRAF*^{V600E}-mutated metastatic melanoma, are small-molecule inhibitors that specifically target the mutated protein, which is present in ~50% of melanomas[1]. Trametinib, another small-molecule inhibitor used for the treatment of melanoma, targets MEK, a downstream effector of BRAF in the hyperactive MAPK pathway[2]. Melanoma mutations may also derive neoantigens which are degradation products of cancer-specific mutated proteins presented by major histocompatibility complex (MHC) proteins. These have been shown to be mediators of durable remissions in all types of cancer immunotherapy: adoptive cell therapy, immune checkpoint blockade and vaccination[3–5].

The tumour microenvironment (TME) is a critical regulator of tumour development, growth, invasion and metastasis, and plays a central role in influencing both tumour immunity and the patient's prognosis. The presence of tumour-infiltrating lymphocytes (TILs) in melanoma tumours, and their localization, composition and density, can influence both the immune response and patient survival[6]. However, tumour progression may still occur in the presence of T cell infiltration — an indication of induced immune evasion. Several mechanisms may underlie this phenomenon, such as the inhibition of tumour-specific T cells, the lack or low levels of antigens or the MHC molecules which present them, the absence of chemoattractants or their receptors on the infiltrating T cells, or suppressor factors secreted by the tumour cells, neighbouring cells or suppressor immune cells in the TME[7,8].

As well as CD4$^+$ and CD8$^+$ T cells, other innate T cells, such as natural killer T cells (NKT cells), γδ T cells and mucosa-associated invariant T cells (MAIT cells), play a role in controlling tumour growth. Furthermore, different innate and adaptive immune cell types can be found in the tumour, including macrophages, dendritic cells (DCs), B cells, mast cells, natural killer cells (NK cells) and neutrophils.

Immune checkpoint inhibitors (ICIs) have demonstrated significant clinical efficacy in metastatic melanoma by reversing effector T cell dysfunction and exhaustion, thereby enhancing their antitumoural properties. This success has led to ICI use as a first-line treatment for melanoma and other cancer types. However,

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Immune checkpoint
An immune system pathway that acts as a 'gatekeeper' of the immune response. Checkpoint receptors are located on the immune cell surface and play a critical role in regulating the balance between immune cell activation and inhibition, resulting in self-tolerance and prevention of the immune system from attacking self-cells indiscriminately.

Tumour-infiltrating lymphocytes
(TILs). Lymphocytes comprising mainly $CD8^+$ and $CD4^+$ T cells, but also containing B cells and natural killer cells (NK cells).

40–60% of patients with melanoma do not achieve a significant therapeutic response and many responders experience tumour relapse[5,9–12]. There may be an overlap between mechanisms that prevent an initial immune response and those that result in resistance to ICI therapy; however, the timing and order of immune alterations within the melanoma will also likely determine whether an initial immune response can occur. A more complete understanding of the mechanisms resulting in immune evasion will be critical to identifying potential therapeutic strategies and using them at the appropriate stage of disease.

In this Review, we provide an overview of recent advances in our understanding of the immune response in melanoma, by focusing on the different mechanisms that effect antitumour immunity. We discuss the role of tumour cell-intrinsic factors in the immune response, how immune cells in the TME interact with the tumour cells, how immune cells function in the tumour as well as the effect of the microbiome on antitumour immunity. We discuss how this information will contribute to better targeting of the immune mechanisms involved in the antitumour immune response, the development of therapeutic modalities, the role of timing and the combinations of different types of treatments for maximum effectiveness.

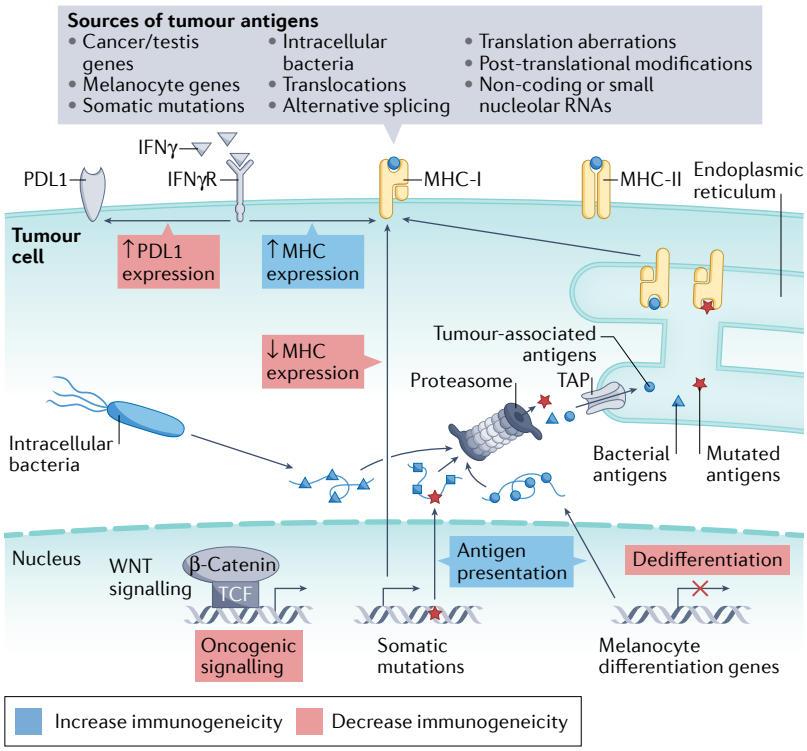

Fig. 1 | **Tumour-intrinsic mechanisms and their effect on the antitumour immune response.** An increase in antitumour immunogenicity (blue) can result from interferon-γ (IFNγ) secretion, leading to increased major histocompatibility complex (MHC) expression and peptide presentation by tumour cells. Upper panel: potential sources of tumour antigens, including antigens that harbour tumour mutations, intracellular bacteria, melanocyte differentiation genes and other tumour-specific alterations. The antigens are processed into peptides by the proteosome and transported by the transporter associated with antigen processing (TAP) from the cytosol into the endoplasmic reticulum for assembly with MHC class I (MHC-I) molecules. Some of these antigens can also be presented by MHC-II. Decrease in immunogenicity (red) can be due to IFNγ secretion, which results in PDL1 expression by tumour cells, mutations in tumour cells that cause the loss of MHC expression or alterations in oncogenic signalling pathways such as WNT–β-catenin. Dedifferentiation and loss of melanocyte differentiation antigens can also result in resistance to immunotherapy.

Tumour cell-intrinsic factors

The antigenicity of tumour cells arises principally as a consequence of their altered genetic, transcriptional and functional landscapes. These tumour cell-intrinsic factors determine the induction and maintenance of a naturally occurring antitumour T cell response, on the one hand, or can lead to resistance to ICIs, on the other (FIG. 1).

Tumour cell antigenicity

Melanomas are highly antigenic tumours, as is evident in cases of spontaneous tumour regression, the high infiltration of T cells and the higher response rates of patients to ICIs. Although antigen-unaware therapies — such as ICIs, adoptive cell therapy of bulk non-selected TILs or the use of lysed tumour cells — are successful in the treatment of melanoma[13,14], it is critical that we understand the sources of tumour cell antigenicity in order to develop antigen-targeted therapies. This goal has led to the identification of tumour-specific antigens expressed only by the tumour cells and tumour-associated antigens, which have elevated levels on tumour cells but are also expressed by normal cells, and their role in the tumour immune response.

Previously, melanoma antigen research focused on tumour-associated antigens derived from wild-type genes that were known to be overexpressed in melanoma cells, for example those encoding melanocyte differentiation antigens (such as tyrosinase, PMEL, MART1 (also known as melan A) or tyrosinase-related protein 1 and 2), or antigens restricted to dispensable tissues such as the testis and placenta (such as the cancer/testis antigen NY-ESO-1 and melanoma-associated antigen (MAGE) family proteins)[13]. Recent studies have revealed that mutated antigens in the tumour can function as immuno-dominant antigens, as they are not expressed in normal tissues and, hence, bypass central tolerance[15,16]. These findings led to efforts to develop advanced methods for mutated peptide identification and understanding how to better predict their presentation on MHC molecules found on cancer cells as well as their immunogenicity (BOX 1).

The mutation rate of melanoma is among the highest from all cancer types[17]; however, despite the therapeutic potential of mutated antigens, they are highly patient-specific, and the number of identified shared mutated antigens, derived from recurrent melanoma mutations, remains low[18]. Similarly, the number of clinically relevant mutated antigens identified in each patient is limited, including only a few mutated antigens per patient, or none at all[19]. Another hurdle in the use of mutated antigens is their heterogeneity within both the tumour and metastatic sites, owing to the heterogeneity in the tumour mutational landscape resulting from tumour immuno-editing and the selection effects of diverse therapeutic approaches. Reduced tumour heterogeneity has been shown to lead to robust immune surveillance

Box 1 | **Antigen identification methods**

For therapeutic purposes, the ideal tumour antigens should be immunogenic and tumour-specific, presented by the tumour cells. Current antigen identification methods rely on these attributes and are based on the physical purification of the antigens using immunopeptidomics[16,25,34,165–167], or on the presence of T cells that recognize them, identified by antigen screens using peptides[168], tandem mini-genes[169,170], cDNA libraries[15,171] or tetramers[172–175]. All of these methods require prior knowledge of the antigen sequences, which are used as the input for antigen identification.

When performing immunopeptidomics, the cells are lysed and the peptide–major histocompatibility complex (MHC) complexes are immunopurified. The peptides are isolated and analysed using mass spectrometry, and the mass spectrometry data used to interpret the peptide sequences. The advantage of this method is that it enables the identification of the antigens presented by the tumour cells, even if no prior immunity exists towards them. However, this method presents a snapshot of the tumour antigens at the dissection time point and is limited by the mass spectrometry detection capabilities.

Screening methods that rely on immunogenicity enable the identification of antigens that had previously induced immunity in the tumour. The main disadvantage is that these antigens might no longer be present in the tumour due to immuno-editing, whereas other presented antigens could be missed if they were not immunogenic. Screens using minimal epitopes, such as tetramers and short peptides, are more prone to bias, as they require the prediction of the screened peptides using MHC-binding prediction algorithms.

A combination of these methodologies can give a broader view of the MHC-presented antigens in the tumour, while compensating for their individual disadvantages, in order to identify targetable tumour antigens[165].

in a melanoma mouse model[20], which could explain the link between tumour heterogeneity and patient survival[21–23].

Our increasing understanding of antigen processing and presentation pathways and the mining of the genomic, transcriptomic and proteomic tumour landscapes is shedding light on the repertoire of presented antigens. Technologies such as whole-genome and whole-exome sequencing, RNA sequencing and ribosome profiling have allowed the identification of antigens derived from mutations (non-synonymous single-nucleotide variants, frameshifts and indels), translocations, alternative splicing (fusions of transcripts, or alternative mRNA or proteasomal splicing events), alternative ribosomal products, post-translational modifications and non-coding or small nucleolar RNAs[14]. Other sources of antigens presented by melanoma cells are endogenous retroviruses[24] and intratumour bacteria[25] (discussed below).

IFNγ responsiveness and signalling

Activation of the interferon-γ (IFNγ) pathway in tumour cells has a dual role in the antitumour immune response, depending on the duration of activation and the presence of IFNγ pathway components. IFNγ signalling has a direct anti-proliferative and pro-apoptotic effect[26–28], can result in the secretion of chemokines such as CXCL9 and CXCL10 (REFS[29–31]), and increases the expression of antigen-processing machinery and surface MHC class I (MHC-I) and MHC-II molecules[32,33]. IFNγ can also alter the repertoire of presented antigens, resulting in higher immunogenicity of the tumour cells[34]. In contrast, chronic exposure of melanoma cells to IFNγ has been shown to result in (PDL1-independent) epigenetic and transcriptomic changes, leading to upregulation of inhibitory T cell receptors and resistance to ICIs[35] (FIG. 1).

As IFNγ induces the expression of PDL1, disruption of this pathway may affect the sensitivity to therapies targeting this immune checkpoint molecule. Indeed, tumour relapse of patients with melanoma, who previously responded to anti-PD1 therapy, was shown to be a result of loss-of-function mutations in the genes encoding Janus kinase 1 (JAK1) and JAK2 (REF.[36]). CRISPR screens, which were designed to identify genes associated with resistance to immunotherapy in mouse and human melanoma cells, have also identified genes encoding components of the IFNγ signalling pathway, including JAK1, JAK2, signal transducer and activator of transcription 1 (STAT1), IFNγ receptor 1 (IFNGR1) and IFNGR2 (REFS[37–39]). Other components identified in these studies include the protein tyrosine phosphatase PTPN2, which reduces the sensitivity to IFNγ receptor signalling[37,39], apelin receptor (APLNR), which interacts with JAK1 and increases the sensitivity of tumour cells to IFNγ[38], and components of the chromatin regulator PBAF complex, which suppress the expression of IFNγ-response genes, resulting in resistance to T cell-mediated killing[39].

Loss of antigen presentation

The effect of IFNγ signalling on antitumour immunity relies on its ability to upregulate MHC-I antigen presentation. Whereas in some MHC-I-deficient tumours, treatment with IFNγ can restore the expression of the antigen-processing machinery and MHC-I expression, in other cases, defects in antigen presentation are irreversible[40]. For example, longitudinal biopsies revealed that loss of MHC-I in some patients with metastatic melanoma was due to loss of the gene encoding β2-microglobulin (*B2M*)[41]. Similarly, mutations in *B2M* resulted in resistance to immunotherapy. Patients with melanoma who received immunotherapy treatments, such as interleukin-2 (IL-2), IFNγ, adoptive TIL therapy or mutated antigen vaccine, lost functional expression of B2M and, as a result, had low MHC-I expression[42,43]. Point mutations, deletions or loss of heterozygosity (LOH) were also observed in patients with melanoma with resistance to ICIs[36,44]. LOH at the *B2M* locus was enriched threefold in non-responders and was associated with lower overall survival in two independent cohorts of patients with melanoma treated with anti-cytotoxic T lymphocyte antigen 4 (CTLA4) or anti-PD1 (REF.[44]).

Genes that decrease the susceptibility of melanoma cells to killing by TILs include *MEX3B*, which encodes a post-transcriptional negative regulator that destabilizes *HLA-A* mRNA leading to reduced levels of MHC-I. Indeed, higher expression of *MEX3B* was found in patients who were non-responsive to anti-PD1 therapy[45].

Although the role of MHC-II expression on melanoma cells remains unclear, it has been suggested for use as a predictive biomarker of response to anti-PD1 therapy. Although defects in MHC-II antigen presentation are less frequent compared with the MHC-I presentation pathway, MHC-II-mediated resistance mechanisms to PD1 inhibition have been reported[46–48]. Additionally, aberrant expression of MHC-II in melanoma was found to attract $CD4^+$ T cells, which dampen $CD8^+$ T cell antitumour responses[49]. Immunogenic tumour-associated and

Natural killer T cells (NKT cells). Members of the family of unconventional T cells that recognize glycolipid antigens in the context of the non-polymorphic major histocompatibility complex class I (MHC-I)-like molecule CD1D. NKT cells are characterized by their capacity to rapidly produce a large amount of immunoregulatory cytokines and may play a role in antitumour immunity, particularly via their secretion of interferon-γ (IFNγ), which cross-activates natural killer cells (NK cells).

Dendritic cells (DCs). Antigen-presenting cells (APCs) that present tumour antigens to $CD4^+$ and $CD8^+$ T cells. Antigen presentation is done efficiently only by mature DCs. DC maturation is affected by different factors in the tumour microenvironment (TME), for example tumour-associated macrophages (TAMs) limit DC maturation and are therefore able to evade the host immune response. In addition, DCs are mediators of immune tolerance and regulatory T cell (T_{reg} cell) expansion.

Natural killer cells
(NK cells). NK cell activation relies on signals derived from multiple activating and inhibitory receptors and does not require antigen specificity. NK cell function is partially complementary to T cells, as NK cells target and lyse major histocompatibility complex class I (MHC-I)-deficient cells and, therefore, play an essential role in cancer immunosurveillance.

Tumour immuno-editing
Immuno-editing that occurs during tumour progression to allow the immune system to initially constrain but later promote tumour development. Initially, the immune system recognizes the transformed cells and eliminates them. Tumour cells that are not eliminated can progress to an equilibrium phase. Edited tumours can then escape the immune system and exhibit unrestrained growth.

specific MHC-II antigens have been previously described (detailed in Cancer Antigenic Peptide Database), and MHC-II mutated peptides have been shown to mediate a $CD4^+$ immune response in patients with melanoma who were vaccinated with personalized mutated antigens[43,50,51].

Oncogenic signalling

In addition to the contribution that genetic alterations make to the antigenic tumour repertoire (for example, mutated and overexpressed tumour antigens), oncogenic signalling can affect tumour immunity via various other mechanisms. Although mechanisms shown in other types of cancer can be also relevant to melanoma, here we limit our review to the most common oncogenic signalling mechanisms previously described in melanoma models and patients with melanoma.

The MAPK signalling pathway plays a critical role in melanoma development and immune evasion, with approximately half of the patient tumours harbouring the $BRAF^{V600E}$-activating mutation. Inhibition of mutated BRAF protein, using vemurafenib, showed increased susceptibility of melanoma cells to T cell-mediated cytotoxicity, without affecting T cell function[52], via increased expression of MHC-I and melanoma differentiation antigens[53,54]. Vemurafenib can also synergize with IFNγ and tumour necrosis factor (TNF) signalling to induce cell-cycle arrest of tumour cells bearing $BRAF^{V600E}$ mutations[55]. BRAF or MEK inhibition also decreased the production of the immunosuppressive factors IL-10, IL-6 or vascular endothelial growth factor (VEGF) by melanoma cells, which, in turn, reduced their suppressive activity on the production of inflammatory cytokines IL-12 and TNF by DCs[56].

Other alterations in melanoma cells that reduce the susceptibility of tumour cells to T cell-mediated tumour killing include loss of *PTEN*, which also correlates with decreased T cell infiltration and inferior outcomes with PD1 inhibitor therapy[57]. Activation of WNT–β-catenin signalling was correlated with reduced T cell infiltration, production of immunosuppressive cytokines, such as IL-10 (REF.[58]), and the prevention of T cell priming to antitumour responses by disrupting the recruitment of DCs expressing the transcription factor BATF3 (REFS[59,60]). Mutations in the gene encoding ATRkinase, frequent in melanoma, have been reported to decrease intratumoural T cell infiltration and expression of immune checkpoints[61]. Recent evidence suggests that tumour dedifferentiation may also play a role in resistance to immunotherapy. For example, loss of the melanocyte differentiation antigen MART1 has been observed in relapsed tumours after adoptive cell therapy[62]. In another example, downregulation of MHC-I expression was described as a hallmark of resistance to PD1 inhibition and was associated with a dedifferentiated phenotype[63].

Immune cell effects on melanoma tumours

Immune checkpoints

The activity of TILs is a major determinant of successful immune surveillance. TILs can recognize antigens presented by the tumour cells, and with the engagement of co-stimulatory factors TIL activation can mediate tumour cell killing and, thus, control tumour growth. On the other hand, TIL activity can be hampered by expressing inhibitory checkpoint molecules. These immune checkpoints and their ligands are diverse in their cell distribution and in their functional role and involvement in the immune response[64,65] (FIG. 2).

The most extensively studied immune checkpoint molecules are PD1 and CTLA4, whose inhibitors are widely used clinically and are the first line of treatment for patients with melanoma[66]. Additional immune checkpoints include lymphocyte activation gene 3 protein (LAG3), T cell immunoglobulin mucin receptor 3 (TIM3; also known as HAVCR2) and T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT). These immune checkpoints have been shown to be upregulated in the $CD8^+$ subset of TILs by single-cell analysis in human melanoma tumour samples[65]. Chronically stimulated $CD8^+$ T cells acquire an 'exhausted' state, characterized by loss of cytolytic activity, reduced cytokine production, reduced proliferation capacity and upregulation of these co-inhibitory receptors. Q9

The use of ICIs has transformed melanoma treatment, with improved overall survival of patients with advanced melanoma. ICIs also serve as the first line of therapy for other cancers, such as non-small cell lung cancer[67]. Although monotherapy using CTLA4 or PD1 blocking antibodies has significantly prolonged the survival of some patients, 40–60% of patients do not respond[12], which has led to the development of combination treatments targeting additional immune checkpoint molecules, such as TIM3 and LAG3.

TIM3 has been shown to bind two ligands: first, galectin 9 is a secreted protein produced by immune cells including mast cells, T cells and antigen-presenting cells (APCs) and by non-immune cells such as fibroblasts. Similar to PDL1, galectin 9 is upregulated by IFNγ and is a part of the negative feedback loop triggering T cell-mediated death. Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is also a TIM3 ligand, expressed by T cells, APCs and tumour cells[68,69]. Q10 Q11

TIM3 and CD39 (also known as ENTPD1) were associated with a $CD8^+$ T cell exhausted state in samples from patients with melanoma. Indeed, a combination of a small molecule (POM-1) that inhibits CD39 and a TIM3 blocking antibody reduced tumour growth in the B16-F10 mouse model of melanoma[70]. In addition, TIM3 expression by NK cells has been associated with NK cell exhaustion in advanced melanoma, and its blockade reversed the exhausted phenotype[71]. Q12

Furthermore, B and T lymphocyte attenuator (BTLA), an immunoglobulin-like molecule expressed by T cells, NK cells and APCs, was shown to be upregulated in NY-ESO-1-specific $CD8^+$ T cells in patients with melanoma. Blockade of BTLA combined with PD1 and TIM3 blockade enhanced NY-ESO-1-specific $CD8^+$ T cell expansion and function[72].

Additional immune checkpoint molecules have been identified, including V-type immunoglobulin domain-containing suppressor of T cell activation (VISTA), adenosine receptor A2A, B7-H3, B7-H4 and killer cell immunoglobulin-like receptors (KIRs), all of which are at different stages of research and clinical development[73–78].

Dynamic changes in immune cells

The interactions between different components of the TME lead to dynamic changes in cell populations, which are critical for determining tumour progression. Melanoma tumours are characterized by relatively high infiltration of immune cells, and are therefore considered an immunogenic malignancy. However, these immune cells can have opposing effects, either initiating or inhibiting the immune response.

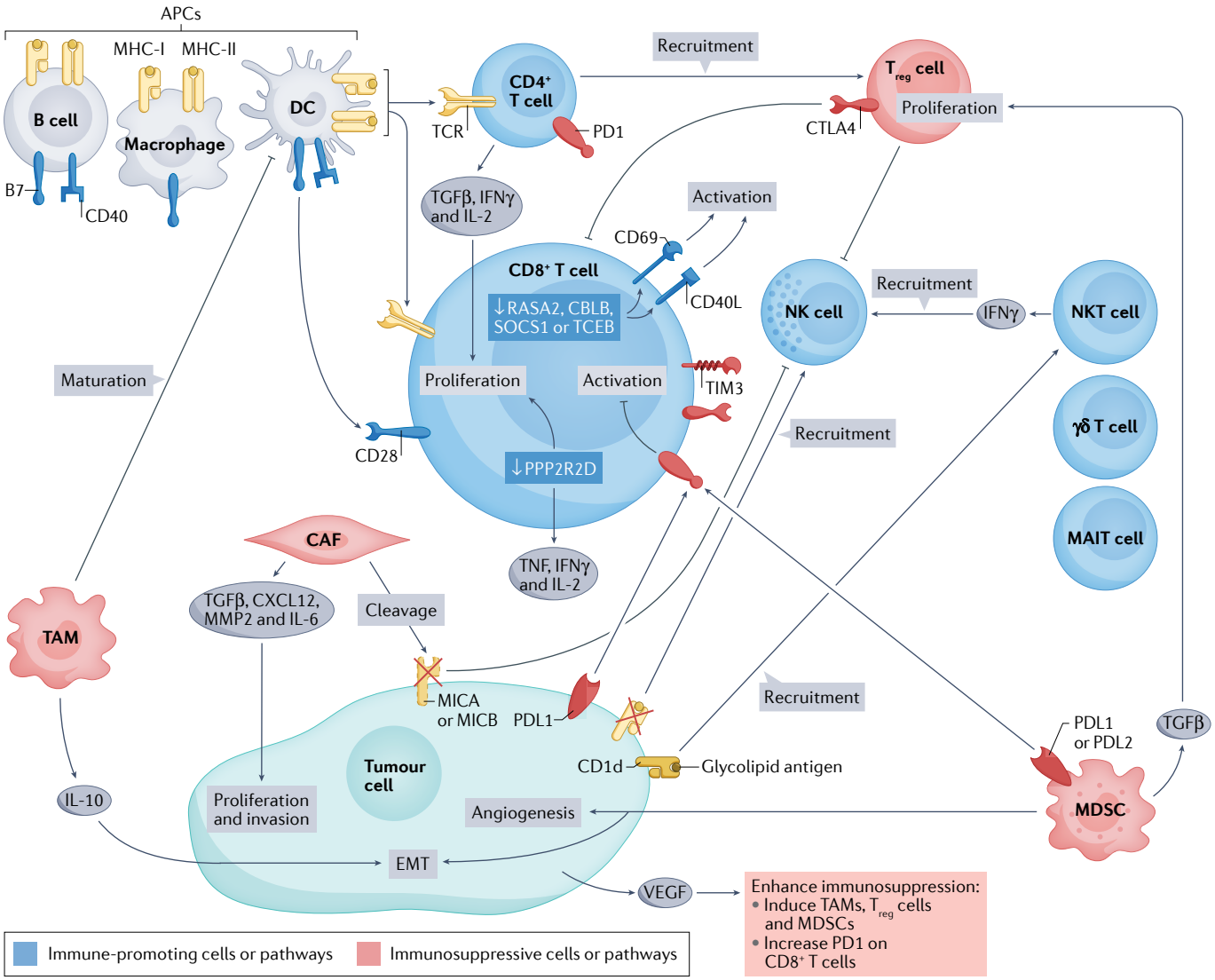

Fig. 2 | **Effect of immune and stromal cells on melanoma tumours.** Immune cytotoxic cell subset includes $CD8^+$ T cells and natural killer cells (NK cells), which can eradicate tumour cells. $CD4^+$ and $CD8^+$ T cells are activated via T cell receptors (TCRs) by antigens presented on major histocompatibility complex class I (MHC-I) and MHC-II by antigen-presenting cells (APCs) and tumour cells. $CD4^+$ T cell secretion of TGFβ, interferon-γ (IFNγ) and interleukin-2 (IL-2) leads to $CD8^+$ T cell proliferation. CRISPR screens have demonstrated that the ablation of Ras GTPase-activating protein 2 (RASA2), CBLB, suppressor of cytokine signalling 1 (SOCS1) or TCEB enhances $CD8^+$ T cell function by increasing expression of the CD69 and CD40L activation markers. In addition, PPP2R2D knockdown enhances $CD8^+$ T cell proliferation and cytokine production. Natural killer T cells (NKT cells) recognize glycolipid antigens presented by CD1D on tumour cells and activate NK cells by IFNγ secretion. Additional non-conventional T cells, such as mucosa-associated invariant T cells (MAIT cells) and γδ T cells, can provide antitumour cytotoxicity. Inhibitory cell subset includes regulatory T cells (T_{reg} cells), which inhibit $CD8^+$ T cells, NK cells, B cells and APCs. Cytotoxic T lymphocyte antigen 4 (CTLA4) expressed by T_{reg} cells competes with CD28 for the binding of B7 ligands on APCs and inhibits $CD8^+$ T cell activation. Another inhibitory immune cell type is myeloid-derived suppressor cells (MDSCs), whose expression of PDL1 and PDL2 inhibits T cell activation by binding to PD1. Furthermore, MDSCs promote T_{reg} cell proliferation in a TGFβ-dependent manner and bolster angiogenesis in the tumour microenvironment (TME) and epithelial–mesenchymal transition (EMT) in tumour cells. Tumour cells can induce the activity of T_{reg} cells, tumour-associated macrophages (TAMs) and MDSCs by secreting vascular endothelial growth factor (VEGF) and enhancing expression of PD1 on $CD8^+$ T cells. Additionally, TAMs limit dendritic cell (DC) maturation and promote EMT by IL-10–Toll-like receptor (TLR) signalling. NK cells are activated by downregulation of MHC-I and expression of MICA and MICB by tumour cells. Cancer-associated fibroblasts (CAFs) can also induce immunosuppression. CAFs cleave the ligand of NK cell-activating receptors on the surface of tumour cells, thereby reducing natural killer group 2D (NKG2D)-mediated cytotoxicity directed against the tumour. In addition, CAFs secrete TGFβ, CXCL12, of matrix metalloproteinase 2 (MMP2) and IL-6, which promote tumour proliferation and invasion. TIM3, T cell immunoglobulin mucin receptor 3.

Immune cell activation in the TME. T cells have a major role in the antitumour immune response. Short hairpin RNA (shRNA) and CRISPR screens in T cells have identified pathways mediating T cell-specific cellular functions, and this research has contributed to drug development and the design of genetically reprogrammed T cell-based therapies. These technologies have been used in both in vivo and in vitro models, identifying positive and negative regulators of T cell receptor (TCR) signalling. An in vivo shRNA screen performed in tumour-bearing mice injected with OT-1 TCR-transgenic $CD8^+$ T cells, which recognize B16 melanoma cells expressing the surrogate tumour antigen ovalbumin, identified the target PPP2R2D, a regulatory subunit of the PP2A phosphatase family. *Ppp2r2d* knockdown enhanced T cell proliferation and cytokine production[79]. A separate genome-scale CRISPR screen performed in primary human $CD8^+$ cells identified several negative regulators of TCR signalling, such as Ras GTPase-activating protein 2 (RASA2), the E3 ubiquitin ligase CBLB, suppressor of cytokine signalling 1 (SOCS1) and the SOCS1 binding partner TCEB2. Ablation of each of the genes encoding these regulators enhanced $CD8^+$ T cell function, by increasing the expression of CD69 and CD40L cell surface markers of early activation, and killing of the target A375 melanoma cell line[80]. These studies reveal that T cells can be genetically modified to have an anti-melanoma tumour activity.

Another aspect in promotion of the immune response in patients with melanoma is the presence of highly organized tertiary lymphoid structures (TLSs) within the tumour, which include B cells, T cells and DCs. These cells form highly specialized immune aggregates surrounding high endothelial venules (HEVs), enabling the recruitment of naive B cells and T cells. Both tumour-infiltrating B cells and TLSs have been shown to promote an immunotherapy response in patients with melanoma[81,82]. The immune mechanisms that are activated within these structures are not fully understood; however, the importance of tumour-associated TLSs has been clearly established.

Innate immune cells, such as NK cells, can also eliminate tumour cells. Binding of the receptor natural killer group 2D (NKG2D) to stress-induced proteins MICA and MICB expressed by tumour cells activates cytotoxic activity of NK cells. Proteolytic shedding of MICA and MICB molecules is associated with tumour progression. Antibodies targeting the site of the proteolytic shedding prevented loss of MICA and MICB, and has been shown to reduce human melanoma metastasis in a humanized mouse model[83].

Further, unconventional innate-like T cells — including γδ T cells[84], NKT cells[85] and MAIT cells[86] — are also important in regulation of tumour immunity. For instance, MAIT cells promote tumours by suppressing T cells and NK cells; this has been demonstrated by injection of mouse melanoma cells into mice with depletion of MHC-I-related protein 1 (MR1), which is required for MAIT cell development and function[87].

Immune cell suppression in the TME. Numerous different cells in the TME, including stromal cells, fibroblasts, regulatory T cells (T_{reg} cells), myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs), suppress immune cell activation. T_{reg} cells are immunosuppressive T cells that can inhibit an antitumour immune response through inhibition of $CD8^+$ T cells, NK cells, B cells and APCs. High levels of T_{reg} cell infiltration in a wide range of cancers, including melanoma, have been associated with recurrence, tumour progression and metastasis[88,89].

Myeloid lineage deletion of general control non-derepressible 2 (*Gcn2*), a gene encoding a stress-response kinase that acts as an environmental sensor controlling transcription and translation in response to nutrient availability, in mice shifted TAM and MDSC phenotypes towards an increased antitumour response, following the injection of B16 melanoma cells into the GCN2-depleted mice. The change in the inhibitory function of these immune suppressor cells was due to pro-inflammatory responses and an increase of IFNγ in $CD8^+$ T cells. GCN2 activity was negatively correlated with antitumour responses and overall survival in patients with melanoma[90].

Among the stromal cells present in the TME, cancer-associated fibroblasts (CAFs) are among the most abundant. These cells have a role in creating the extracellular matrix (ECM) structure surrounding the tumour cells; they also play a role in tumorigenesis. It has been shown using human melanoma-associated CAFs that the secretion of matrix metalloproteinases (MMPs) leads to a decrease in the levels of the NKG2D ligands MICA and MICB, thus inhibiting tumour cell susceptibility to NK cell-mediated killing[91]. Additionally, the ECM and proteolytic products derived from ECM remodelling might also have a crucial role in resistance to immunotherapy, as ECM remodelling, collagen deposition and mechanical forces can regulate immune cell migration and activation[92–94]. From the above, it is clear that in cases of immunogenic tumours, such as melanoma, there is a constantly shifting equilibrium between different arms of the immune system leading to tumour recognition and elimination. In parallel, tumour evolution also comprises immuno-editing mechanisms allowing tumour cell escape from immune surveillance and antitumour immunity.

Based on these findings, targeting immunosuppressive cellular populations has become an attractive strategy for therapeutic intervention, principally by inhibiting their recruitment into the TME and administration of co-stimulatory molecules to enhance T cell activation[95].

Metabolic regulation of tumour immunity

Both tumour cells and immune cells compete for resources in the TME, where challenges such as hypoxia, oxidative stress and nutrient deprivation are commonly encountered. Aerobic glycolysis is required for T cell activation and tumour cell proliferation. In T cells, glucose is required for IFNγ signalling and the cytotoxicity function of these cells[96]. As tumour cell proliferation is also dependent on aerobic glycolysis, a phenomenon termed 'the Warburg effect', this may result in competition between immune cells and tumour cells for glucose consumption in the TME[97]. Tumour cells and

immune cells also utilize the non-essential amino acid glutamine, necessary for cellular proliferation as well as metabolite production and fatty acid synthesis. In T cells, glutamine controls mTOR activation and regulates T cell function and differentiation[98]. Additionally, tryptophan, an essential amino acid, is catabolized through the kynurenine pathway, generating metabolites that suppress T cell proliferation[99]. Tryptophan depletion activates GCN2, which inhibits T cell function. Furthermore, amino acid deprivation impairs T helper 17 (T_H17) cell differentiation and promotion of T_{reg} cell development[100,101].

In addition to nutrients, abnormal features of tumour vasculature can create hypoxic regions, leading to enhanced glycolytic activity and lactate production. The increase in lactate acidifies the TME, influencing the antitumour immune response[102]. In melanoma, lactate has been shown to suppress NK cell and T cell responses in the TME[103].

The nutrient competition between the tumour and the immune cells provides yet another means by which tumour cells can evade the immune response[104]. This principle needs to be taken into account when studying antitumour immunity.

Involvement of the microbiome

The tumour microbiome

There have been numerous reports of bacteria within tumours, dating back nearly 100 years[105]. There is a large gap of knowledge regarding the intratumour microbiome in melanoma tumours. Most studies have been performed on tumours in the aerodigestive tract.

A recent study revealed that a broad range of tumours, including melanoma, possess microbiomes, suggesting that tumour microbiomes are more prevalent than previously thought. This study demonstrated the presence of microbes within tumours not directly associated with the aerodigestive tract (defined as the airway, pulmonary tract and upper digestive tract), which are, therefore, less prone to contain commensal organisms. These microbes appear to be tumour type-specific, suggesting that they have tumour-specific roles[106].

Box 2 | **Microbiome species identification methods**

The intratumour microbiome can be identified using several methods, during which the handling of the tumour must be performed in sterile conditions, to avoid contamination. The most common method for bacterial identification is 16S rRNA gene sequencing, as 16S rRNA is present only in prokaryotic cells. This method, which has been used to compile most of the data in the Human Microbiome Project (HMP)[176], requires the processing of the sample DNA, PCR-based amplification of the hypervariable regions (also known as V regions), and sequencing and comparison with reference databases, such as Greengenes[177], SILVA[178] and the Ribosomal Database Project[179]. Sequencing of four or five V regions is highly specific for most bacteria[106,180]. An alternative approach is whole-genome shotgun sequencing, which uses random primers to sequence overlapping regions of the genome. This method requires metagenomics analyses, and enables more accurate taxonomic classification, through determination of the gene composition of the bacteria[181]. An additional option for identifying specific bacteria is through their isolation and growth in culture media, followed by taxonomic profiling using metagenomics analyses. An advance in mass spectrometry techniques (matrix-assisted laser desorption ionization–time of flight mass spectrometry) has been used for cultivatable bacterial species identification due to its high diagnostic accuracy, robustness, reliability and rapid turn-around time[182,183]. However, not all bacterial species can be successfully cultured, limiting the use of this method.

Different mechanisms have been described by which microbes can enter tumours, such as breaches in mucosal barriers, giving luminal bacteria access to the epithelium[107]. Different factors, such as diet, may influence the bacterial composition within tumours. For example, a diet rich in grains and fibres has been correlated with a lower risk for *Fusobacterium nucleatum*-positive colorectal cancer[108].

The intratumour microbiome has been shown to have various functions affecting tumour progression and the response to therapy, including the direct facilitation of tumorigenesis through increased mutagenesis, the regulation of oncogenic pathways or the modulation of the host immune system[109]. In addition, several studies have suggested that the microbiome affects the efficiency of chemotherapeutic drugs used to treat patients with cancer. For example, intratumour bacteria have been shown to increase the drug resistance of tumours for gemcitabine, a chemotherapeutic agent used to treat patients with pancreatic ductal adenocarcinoma (PDAC)[110]. Also, *F. nucleatum* has been shown to promote chemotherapy resistance in colorectal cancer through Toll-like receptor (TLR), microRNAs and autophagy pathways[111].

Influence on the immune response

A role for the gut microbiome in antitumour responses and response to ICIs has been demonstrated in preclinical mouse models and patients with various cancers, including melanoma[112–118]. Specific bacteria were found to correlate with better or worse response to ICIs, and generally a more diverse gut microbiome was correlated with better response to ICIs. Recent clinical trials have demonstrated that faecal microbiota transplantation from donors who previously responded to anti-PD1 therapy to patients with metastatic melanoma who were refractory to anti-PD1 therapy overcame resistance, implying that modulation of the gut microbiome could be developed as a melanoma therapy[119,120]. This effect can be explained by the gut microbiome promoting DC infiltration into tumours, which results in T_H1 cell activation via IL-12 cytokine secretion and $CD8^+$ T cell immune responses[121,122].

Efforts to sequence individual microbes and the human microbiome have provided insight into their influence on health and disease (BOX 2). The use of recently developed computational tools helps minimize the problem of contaminants. These tools are also able to identify cancer-associated microbiomes within human sequencing data sets[123]. Beyond sequencing, microscopy and flow cytometry-based approaches are proving to be useful tools for the detection and study of tumour-associated microbiomes. Bacteria within diverse tumour types may affect both the TME and tumour immunity. RNA sequencing data from The Cancer Genome Atlas (TCGA) have revealed that intratumour gut microbiota can modulate chemokine levels and affect $CD8^+$ T cell infiltration in melanoma, influencing patient survival[124].

On the other hand, many of the bacterial effects in the TME are immunosuppressive. Distinct bacteria in PDAC tumours promote suppressive monocytic cellular differentiation via TLR ligation, leading to T cell anergy[125].

Binding of the *F. nucleatum* Fap2 protein to TIGIT has been shown to inhibit the killing of melanoma tumour cells by NK cells and tumour-infiltrating T cells[126]. The occurrence of *F. nucleatum* is also negatively associated with the presence of TILs in patients with colorectal cancer with high microsatellite instability[127].

A recent study using human melanoma tumours detected bacterial peptide presentation by MHC molecules on both tumour cells and APCs. The MHC-presented bacterial peptides increased the antigenicity of the tumour cells, as well as the response of the TILs to the presenting tumour cells. Bacterial peptides offer an additional type of tumour antigen that could be used as potential targets for immunotherapy in patients with cancer[25].

Translating mechanisms to treatments

Melanoma has long been a model for studying and developing immunotherapy treatments, due to the substantial involvement of immune cells in the melanoma TME. Different treatments have been designed to target different components of the TME, including tumour cell intrinsic and extrinsic factors (see above). Although some of these therapies have been clinically successful in melanoma — such as ICIs, adoptive cell therapy and antigen-specific vaccines — not all patients respond to the selected course of treatment, or they relapse due to tumour evasion of the immune response. Selecting the best targets for each patient can present a substantial challenge, as this requires consideration of the genetic, transcriptional, epigenetic and antigenic milieu of the tumour cells, as well as the other immune and non-immune cellular components of the tumour. Although a combination of treatments targeting multiple components may provide greater efficacy, optimizing the best combination will be necessary to maximize induction of the immune response while minimizing adverse effects and the chance for resistance.

In order to understand the effect of the immune system on the tumour, the first question that should be asked is whether there has been a prior antitumour immune response. If such a response occurred, boosting the immune system or preventing immune inhibition would potentially lead to an antitumour immune response. The most straightforward indicator of a pre-existing antitumour immune response may be the presence of T cells within the TME[128]. In patients with melanoma who received anti-PD1 therapy, the presence of T cells in pretreatment biopsies was associated with response to therapy[129,130]; however, this correlation was not found in another patient cohort[131]. Whereas the presence of tumour-infiltrating T cells may not specifically indicate the presence of an immune response, the use of markers for cytotoxic activity can be more informative, such as the cytolytic activity score, which relies on the expression of granzyme A and perforin 1 (REF.[132]). Similarly, the presence and frequency of specific T cell states predicting survival and response to ICIs, such as the frequency of $TCF7^{+}CD8^{+}$ T cells[133], can also be used as a biomarker for a pre-existing immune response.

If prior immunity exists, the use of ICIs can be beneficial for reversing the local immunosuppressive effects of tumour-specific T cells, or for inducing and recruiting tumour-specific T cells from the periphery[134]. The most direct biomarker for response to ICIs is the expression of immune checkpoint molecules, such as PDL1, in the TME[135]. However, PDL1 expression does not necessarily predict an antitumour immune response, as some patients with PDL1-positive tumours failed to respond to therapy and some patients with PDL1-negative tumours benefited from ICI therapy[136,137]. Other markers for response to ICIs in melanoma have previously been described, such as the IFNγ signature[138,139], the presence of T cells in the tumour[130] and the mutational load or predicted neoantigen load[140–142]. Nevertheless, these biomarkers have not demonstrated robust predictive power in all cohorts tested and are not specific for the ICI response, as they could indicate prior immune responses to the tumour. Another biomarker for ICI responses is the presence of TLSs[82]. These structures, comprising T cells, B cells, DCs and other APCs, may be involved in priming and activation of T cells within the TME, which may explain their role in antitumour T cell responses and responses to immunotherapy. Compared with other biomarkers, the presence of TLSs indicates an ongoing antitumour immune response and provides information on dynamic interactions between multiple cell types, and could potentially be a more inclusive biomarker. Further studies are required to determine the role of TLSs in melanoma and other tumours. Understanding the different interactions that occur within the TLSs, as well as the mechanism of TLS formation, may suggest ways to induce TLS formation in non-immunogenic tumours. Identifying biomarkers to monitor tumour-specific antigen presentation and the engagement of T cells with APCs in TLSs may provide insight into the function of T cells and APCs in controlling tumour progression.

In ICI-sensitive tumours, the combination of ICIs with treatments that increase tumour cell antigenicity can improve the response to ICIs, by removing inhibitory effects on cytotoxic cells or by increasing the tumour-specific antigens that they recognize. Different approaches can be used to increase tumour antigenicity, such as activation of the IFNγ pathway and the antigen presentation pathway, which both induce immunogenic tumour cell death, and/or inhibition of oncogenic signalling pathways[143–145], or enhancement of tumour antigen presentation by increasing APC infiltration and activity[146,147]. To further increase the effectiveness of existing tumour-specific T cells, ICIs could be combined with antigen-specific vaccines to promote expansion of T cells reactive to tumour antigens[43,50,51,148].

If tumours showed prior immunity but are not sensitive to ICIs, this could suggest that the relevant checkpoint molecule is not expressed by the tumour and that other inhibitors targeting other checkpoint molecules are required. Alternatively, the tumour may not present immunogenic antigens, or there may be a lack of T cells that recognize the tumour antigens. A possible strategy in such cases would be to treat with BRAF or MEK targeted therapy prior to or concurrent with ICI therapy, as this may increase antigen expression; these types of regimens have proven efficacy in some clinical trials[143–145], although toxicity can be

Pattern-recognition receptors
Receptors that are expressed by innate immune cells and recognize molecules expressed on the surface of pathogens, apoptotic host cells and damaged senescent cells. These receptors induce immuno-protective effects, such as anti-infection and antitumour effects, and participate in initiation of the immune response.

an issue[146,149,150]. Another strategy would be to vaccinate the patient either with tumour-specific antigens or T cells engineered to express TCRs that target tumour-specific MHC-presented antigens, thereby increasing tumour immunogenicity. Engineered T cells expressing TCRs that recognize tumour antigens presented by the tumour allow targeting of a large repertoire of antigens[151]. Similarly, engineering T cells to eliminate the expression of checkpoint molecules[152] or using treatments that reduce T cell inhibitory factors other than PDL1–PD1 and CTLA4 can increase tumour-specific T cell function.

An alternative source of antigenicity can be derived from the tumour and gut microbiome. As noted above, faecal microbiota transplantation from ICI therapy responders to patients who did not respond to ICI therapy can increase therapeutic potency[119,120]. Other types of interventions in the gut and tumour microbiome can be used to increase both gut microbiome diversity and bacterial species correlated with better responses to ICIs; for example, by modulating the patient's diet or using specific drugs[153]. Similarly, inducing entry of immunogenic bacteria into the tumour could potentially induce innate and adaptive immune responses at the tumour site. Q20 Presentation of bacterial peptides by tumour cells can increase tumour cell antigenicity and the TIL response towards the cells that present the peptides[25].

In tumours showing prior immunity, regardless of their ability to respond to ICIs, treatments that reduce inhibitory effects of cells in the TME or activate T cell activity can be combined with the treatments suggested above. For example, reducing the inhibitory effect of T_{reg} cells, MDSCs, TAMs and CAFs, and the signalling pathways between the different cell types, can reduce the inhibition of T cells and NK cells. Treating patients with different cytokines and chemokines that promote T cell activation and migration of immune cells to the tumour can lead to an antitumour immune activation. For example, treatment with IL-2, IFNγ, CXCL9 and CXCL10 has been associated with increased infiltration of T cells into different tumour types, including melanoma[154–157]. Similarly, tumour antigenicity can be enhanced by activating pattern-recognition receptors, as demonstrated by a synthetic CpG oligonucleotide stimulating TLR9 and inducing a broad immune activation in the TME when combined with ICI treatment in patients with melanoma[158].

Lack of prior immunity in the tumour can result from the loss or absence of antigens, or loss of antigen presentation molecules, such as B2M or MHC molecules. Q21 Whereas the loss or low expression of some of the components of the antigen presentation pathway can be compensated for or restored, and reduced tumour cell antigenicity can be increased using treatments that were discussed previously, treatments in tumours that have loss of MHC molecules cannot rely on TCR–peptide–MHC recognition. As NK cells can recognize tumour cells that have low MHC expression, different treatments that increase NK cell cytotoxicity and recognition of tumour cells can be applied[159]. Similarly, other unconventional T cells, such as NKT cells, γδ T cells and MAIT cells, which are not restricted to MHC recognition, can provide antitumour cytotoxicity[160]. Compared with cytotoxic T cells, less is known regarding relevant biomarkers for NK cell and unconventional T cell functionality in the tumour. Identifying such biomarkers should allow NK cells and unconventional T cells to be used to promote antitumour immunity in different patients. Another treatment approach that does not rely on antigen presentation is the use of chimeric antigen receptor (CAR)-T cells. Although anti-CD19 CARs have proven to be a successful treatment for haematological malignancies, identifying selective targets for solid tumours, including melanoma, is more challenging[161]. In addition to the use of CAR-T cells, recent studies have focused on utilizing other engineered immune cells (such as NK cells[162] and macrophages[163]) to target cancer cells in a similar manner as do CAR-T cells[164] (FIG. 3).

Further work is required to identify biomarkers for treatment selection in individual patients with melanoma and other cancers, and also for monitoring the tumour immune response, particularly with regard to the presentation of antigens and engagement of T cells and APCs, which should help identify steps that could be successfully modulated therapeutically. Similarly, studies are needed to understand the involvement of the different cell types in the TME. As different treatments change the contexture of the tumour, the selection of the subsequent treatment requires a further investigation of the tumour status and the relevant resistance mechanisms.

Conclusions and perspective

Melanoma is a highly immunogenic tumour due to its mutational burden and therefore the immune response to this tumour has been intensively studied. However, many gaps remain in our knowledge of the melanoma cell-autonomous factors that play a role in melanoma cell immunogenicity and how these factors affect the immune response. A substantial challenge in the field is selecting a potent target that will lead to immune activation. This requires the consideration of multiple properties of the tumour cells such as their transcriptional, translational, epigenetic, proteomic and antigenic landscapes. These would need to be assessed in parallel, their data integrated and considered under different stress microenvironments, and intratumour heterogeneity also accounted for. Importantly, so far, the immunogenicity of each of the identified MHC-presented neoantigens has been assessed in an individual manner. However, it is as yet unknown how concomitant presentation of several categories of immunogenic MHC-presented antigens affects the immune response. Do these act synergistically? Or, alternatively, does the simultaneous presentation of several immunogenic targets dampen their immunogenicity, thus revealing a new layer of intratumour heterogeneity? Additionally, an in-depth understanding of the immune cell function within the tumour is lacking and should be investigated; for example, gaps remain in our knowledge of the intratumour role of TLSs, and of the role of MHC-II presentation and its interaction with $CD4^+$ T cells, such as T_{reg} cells. We also lack tools to translate insights from basic research into systems that facilitate better therapeutic decision-making, and for the development of novel therapies. Here, we have reviewed recent research and treatment strategies that can be used to drive future therapeutic innovation, and

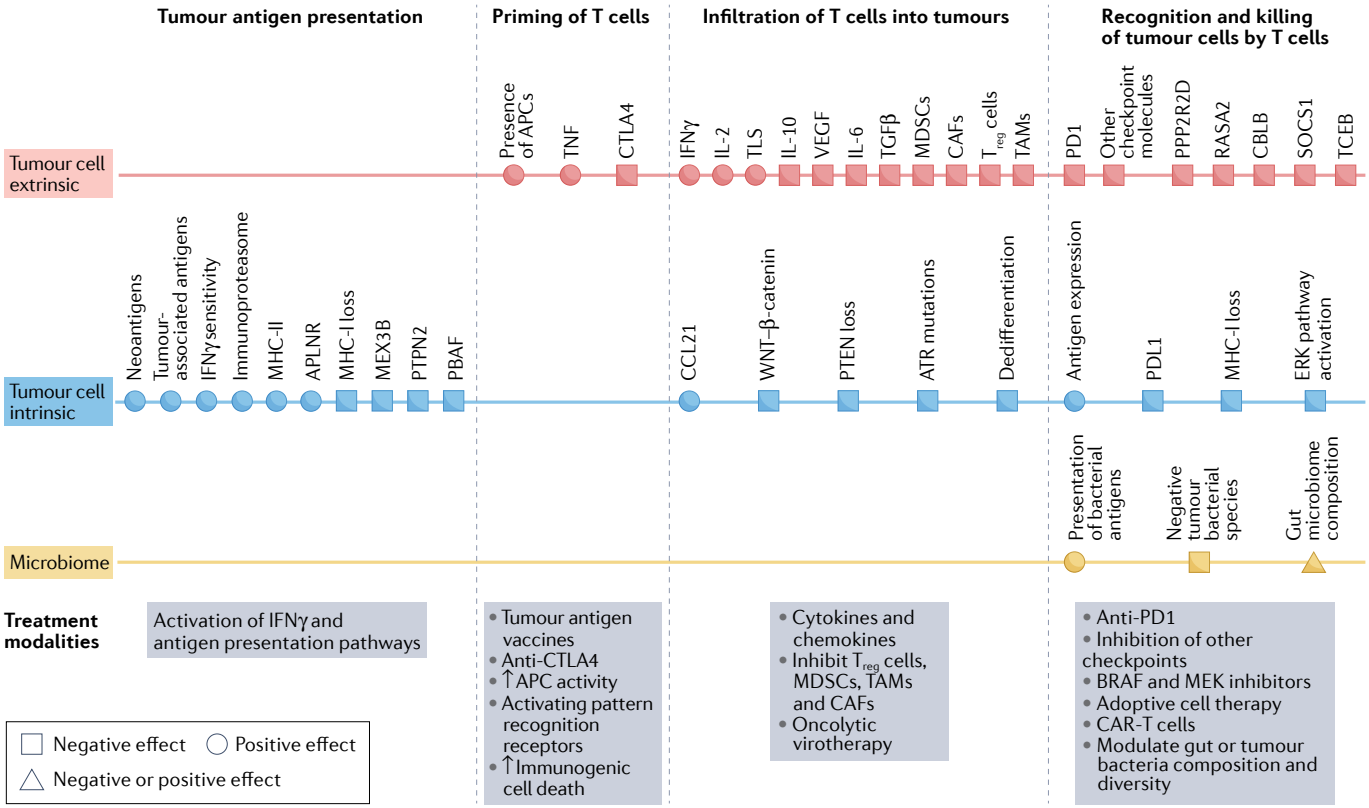

Fig. 3 | **Summary of mechanisms affecting antitumour immunity in melanoma and potential therapeutic modalities.** Different stages of T cell activation: antigen presentation, T cell priming, T cell infiltration and T cell-mediated killing of tumour cells. Various factors that affect immune response in each stage indicated in each row, divided into tumour cell intrinsic (blue) and extrinsic (red) factors or gut/tumour microbiome (yellow). Symbols represent type of effect on antitumour immunity: negative (square), positive (circle) or positive and/or negative (triangle). Different possible therapeutic modalities directed towards mechanisms and/or factors indicated in each stage shown in grey boxes. APC, antigen-presenting cell; CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; CTLA4, cytotoxic T lymphocyte antigen 4; IFNγ, interferon-γ; IL-2, interleukin-2; MDSC, myeloid-derived suppressor cell; MHC-I, major histocompatibility complex class I; RASA2, Ras GTPase-activating protein 2; SOCS1, suppressor of cytokine signalling 1; TAM, tumour-associated macrophage; TLS, tertiary lymphoid structure; TNF, tumour necrosis factor; T_{reg} cell, regulatory T cell; VEGF, vascular endothelial growth factor.

for developing different therapeutic combinations to be used in the clinic or in preclinical studies.

In order to further advance our understanding of cancer immunotherapy and to enable more effective use of the widely available data in the field, we encourage the development of models for optimizing scheduling and administration routes for available therapies, as well as integration of different therapeutic approaches that activate different arms of the immune system. Taking into consideration the effect tumour cell changes have on intratumour and metastatic heterogeneity, and the varying immune response in each niche, will allow the targeting of tumours in a more systematic manner. Monitoring these changes and responding to them with relevant therapeutic methods should help facilitate the elimination of tumour cells, or at least transform melanoma and other cancers from acute into managed, chronic conditions.

Regarding future directions, recent studies have demonstrated that modulation of the microbiome has considerable therapeutic potential in relation to antitumour immunity. Integrating currently available data and further studying the bacterial effect on the immune TME, together with the ability of bacteria to target specific tumours, should provide unique therapeutic options. Furthermore, advances in genome screening methods may help reveal more complex immune phenotypes beyond proliferation and stimulation, and broaden our understanding of immune cell interactions affecting the TME.

Although we have focused on melanoma, some of the immune mechanisms and treatments designed to target these tumours might also be relevant in other types of cancers. However, more research needs to be performed to determine which immune mechanisms are pan-cancer or cancer type-specific.

1. Cancer Genome Atlas Network. Genomic classification of cutaneous melanoma. *Cell* **161**, 1681–1696 (2015).
2. Long, G. V. et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. *Lancet* **386**, 444–451 (2015).
3. Salgaller, M. L., Weber, J. S., Koenig, S., Yannelli, J. R. & Rosenberg, S. A. Generation of specific anti-melanoma reactivity by stimulation of human tumor-infiltrating lymphocytes with MAGE-1 synthetic peptide. *Cancer Immunol. Immunother.* **39**, 105–116 (1994).
4. Blass, E. & Ott, P. A. Advances in the development of personalized neoantigen-based therapeutic cancer vaccines. *Nat. Rev. Clin. Oncol.* **18**, 215–229 (2021).

5. Callahan, M. K. et al. Nivolumab plus ipilimumab in patients with advanced melanoma: updated survival, response, and safety data in a phase I dose-escalation study. *J. Clin. Oncol.* **36**, 391–398 (2018).
6. Fridman, W. H., Pages, F., Sautes-Fridman, C. & Galon, J. The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* **12**, 298–306 (2012).
7. Gajewski, T. F. Failure at the effector phase: immune barriers at the level of the melanoma tumor microenvironment. *Clin. Cancer Res.* **13**, 5256–5261 (2007).
8. Anichini, A., Vegetti, C. & Mortarini, R. The paradox of T-cell-mediated antitumor immunity in spite of poor clinical outcome in human melanoma. *Cancer Immunol. Immunother.* **53**, 855–864 (2004).
9. Larkin, J. et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* **373**, 23–34 (2015).
10. Robert, C. et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **372**, 2521–2532 (2015).
11. Wolchok, J. D. et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N. Engl. J. Med.* **377**, 1345–1356 (2017).
12. Larkin, J. et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N. Engl. J. Med.* **381**, 1535–1546 (2019).
13. Tran, E., Robbins, P. F. & Rosenberg, S. A. 'Final common pathway' of human cancer immunotherapy: targeting random somatic mutations. *Nat. Immunol.* **18**, 255–262 (2017).
14. Haen, S. P., Loffler, M. W., Rammensee, H. G. & Brossart, P. Towards new horizons: characterization, classification and implications of the tumour antigenic repertoire. *Nat. Rev. Clin. Oncol.* **17**, 595–610 (2020).
15. Lu, Y. C. et al. Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression. *J. Immunol.* **190**, 6034–6042 (2013).
16. Kalaora, S. et al. Combined analysis of antigen presentation and T-cell recognition reveals restricted immune responses in melanoma. *Cancer Discov.* **8**, 1366–1375 (2018).
17. Lawrence, M. S. et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* **499**, 214–218 (2013).
18. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69–74 (2015).
19. Yamamoto, T. N., Kishton, R. J. & Restifo, N. P. Developing neoantigen-targeted T cell-based treatments for solid tumors. *Nat. Med.* **25**, 1488–1499 (2019).
20. Wolf, Y. et al. UVB-induced tumor heterogeneity diminishes immune response in melanoma. *Cell* **179**, 219–235.e21 (2019).
21. Reuben, A. et al. Genomic and immune heterogeneity are associated with differential responses to therapy in melanoma. *NPJ Genom. Med.* https://doi.org/10.1038/s41525-017-0013-8 (2017).
22. Lin, Z. et al. Intratumor heterogeneity correlates with reduced immune activity and worse survival in melanoma patients. *Front. Oncol.* **10**, 596493 (2020).
23. Williams, J. B. et al. Tumor heterogeneity and clonal cooperation influence the immune selection of IFN-γ-signaling mutant cancer cells. *Nat. Commun.* **11**, 602 (2020).
24. Schiavetti, F., Thonnard, J., Colau, D., Boon, T. & Coulie, P. G. A human endogenous retroviral sequence encoding an antigen recognized on melanoma by cytolytic T lymphocytes. *Cancer Res.* **62**, 5510–5516 (2002).
25. Kalaora, S. et al. Identification of bacteria-derived HLA-bound peptides in melanoma. *Nature* **592**, 138–143 (2021).
26. Bach, E. A., Aguet, M. & Schreiber, R. D. The IFNγ receptor: a paradigm for cytokine receptor signaling. *Annu. Rev. Immunol.* **15**, 563–591 (1997).
27. Paucker, K., Henle, W. & Cantell, K. Quantitative studies on viral interference in suspended L cells. 3. Effect of interfering viruses and interferon on growth rate of cells. *Virology* **17**, 324–334 (1962).
28. Sucker, A. et al. Acquired IFNγ resistance impairs anti-tumor immunity and gives rise to T-cell-resistant melanoma lesions. *Nat. Commun.* https://doi.org/10.1038/ncomms15440 (2017).
29. Cole, K. E. et al. Interferon-inducible T cell α chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *J. Exp. Med.* **187**, 2009–2021 (1998).
30. Farber, J. M. A macrophage messenger-RNA selectively induced by γ-interferon encodes a member of the platelet factor-IV family of cytokines. *Proc. Natl Acad. Sci. USA* **87**, 5238–5242 (1990).
31. Luster, A. D., Unkeless, J. C. & Ravetch, J. V. γ-Interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. *Nature* **315**, 672–676 (1985).
32. Basham, T. Y. & Merigan, T. C. Recombinant interferon-γ increases HLA-DR synthesis and expression. *J. Immunol.* **130**, 1492–1494 (1983).
33. King, D. P. & Jones, P. P. Induction of Ia and H-2 antigens on a macrophage cell line by immune interferon. *J. Immunol.* **131**, 315–318 (1983).
34. Kalaora, S. et al. Immunoproteasome expression is associated with better prognosis and response to checkpoint therapies in melanoma. *Nat. Commun.* **11**, 896 (2020).
35. Benci, J. L. et al. Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. *Cell* **167**, 1540–1554.e12 (2016).
36. Zaretsky, J. M. et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N. Engl. J. Med.* **375**, 819–829 (2016).
37. Manguso, R. T. et al. In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature* **547**, 413–418 (2017).
38. Patel, S. J. et al. Identification of essential genes for cancer immunotherapy. *Nature* **548**, 537–542 (2017).
39. Pan, D. et al. A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing. *Science* **359**, 770–775 (2018).
Together with Manguso et al. (2017) and Patel et al. (2017), this paper describes CRISPR screens that identify tumour-intrinsic mechanisms of resistance to immunotherapy using in vitro co-culture of melanoma cells and tumour-specific T cells.
40. D'Urso, C. M. et al. Lack of HLA class I antigen expression by cultured melanoma cells FO-1 due to a defect in B2m gene expression. *J. Clin. Invest.* **87**, 284–292 (1991).
41. Sucker, A. et al. Genetic evolution of T-cell resistance in the course of melanoma progression. *Clin. Cancer Res.* **20**, 6593–6604 (2014).
42. Restifo, N. P. et al. Loss of functional β2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. *J. Natl Cancer Inst.* **88**, 100–108 (1996).
43. Sahin, U. et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* **547**, 222–226 (2017).
44. Sade-Feldman, M. et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat. Commun.* **8**, 1136 (2017).
45. Huang, L. et al. The RNA-binding protein MEX3B mediates resistance to cancer immunotherapy by downregulating HLA-A expression. *Clin. Cancer Res.* **24**, 3366–3376 (2018).
46. Rodig, S. J. et al. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aar3342 (2018).
47. Johnson, D. B. et al. Melanoma-specific MHC-II expression represents a tumour-autonomous phenotype and predicts response to anti-PD-1/PD-L1 therapy. *Nat. Commun.* **7**, 10582 (2016).
48. Johnson, D. B. et al. Tumor-specific MHC-II expression drives a unique pattern of resistance to immunotherapy via LAG-3/FCRL6 engagement. *JCI Insight* https://doi.org/10.1172/jci.insight.120360 (2018).
49. Donia, M. et al. Aberrant expression of MHC class II in melanoma attracts inflammatory tumor-specific CD4+ T-cells, which dampen CD8+ T-cell antitumor reactivity. *Cancer Res.* **75**, 3747–3759 (2015).
50. Ott, P. A. et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* **547**, 217–221 (2017).
51. Hu, Z. T. et al. Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma. *Nat. Med.* **27**, 515–525 (2021).
52. Boni, A. et al. Selective $BRAF^{V600E}$ inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. *Cancer Res.* **70**, 5213–5219 (2010).
53. Frederick, D. T. et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin. Cancer Res.* **19**, 1225–1231 (2013).
54. Sapkota, B., Hill, C. E. & Pollack, B. P. Vemurafenib enhances MHC induction in $BRAF^{V600E}$ homozygous melanoma cells. *Oncoimmunology* https://doi.org/10.4161/onci.22890 (2013).
55. Acquavella, N. et al. Type I cytokines synergize with oncogene inhibition to induce tumor growth arrest. *Cancer Immunol. Res.* **3**, 37–47 (2015).
56. Sumimoto, H., Imabayashi, F., Iwata, T. & Kawakami, Y. The BRAF–MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. *J. Exp. Med.* **203**, 1651–1656 (2006).
57. Peng, W. et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. *Cancer Discov.* **6**, 202–216 (2016).
58. Yaguchi, T. et al. Immune suppression and resistance mediated by constitutive activation of Wnt/β-catenin signaling in human melanoma cells. *J. Immunol.* **189**, 2110–2117 (2012).
59. Spranger, S., Bao, R. Y. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).
60. Spranger, S., Dai, D., Horton, B. & Gajewski, T. F. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy. *Cancer Cell* **31**, 711–723.e4 (2017).
61. Chen, C. F. et al. ATR mutations promote the growth of melanoma tumors by modulating the immune microenvironment. *Cell Rep.* **18**, 2331–2342 (2017).
62. Mehta, A. et al. Immunotherapy resistance by inflammation-induced dedifferentiation. *Cancer Discov.* **8**, 935–943 (2018).
63. Lee, J. H. et al. Transcriptional downregulation of MHC class I and melanoma de-differentiation in resistance to PD-1 inhibition. *Nat. Commun.* **11**, 1897 (2020).
64. Paijens, S. T., Vledder, A., de Bruyn, M. & Nijman, H. W. Tumor-infiltrating lymphocytes in the immunotherapy era. *Cell Mol. Immunol.* **18**, 842–859 (2021).
65. Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **352**, 189–196 (2016).
66. Waldman, A. D., Fritz, J. M. & Lenardo, M. J. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat. Rev. Immunol.* **20**, 651–668 (2020).
67. Ribas, A. & Wolchok, J. D. Cancer immunotherapy using checkpoint blockade. *Science* **359**, 1350–1355 (2018).
68. Yang, R. et al. Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. *Nat. Commun.* **12**, 832 (2021).
69. Wolf, Y., Anderson, A. C. & Kuchroo, V. K. TIM3 comes of age as an inhibitory receptor. *Nat. Rev. Immunol.* **20**, 173–185 (2020).
70. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **176**, 404 (2019).
71. da Silva, I. P. et al. Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade. *Cancer Immunol. Res.* **2**, 410–422 (2014).
72. Fourcade, J. et al. $CD8^+$ T cells specific for tumor antigens can be rendered dysfunctional by the tumor microenvironment through upregulation of the inhibitory receptors BTLA and PD-1. *Cancer Res.* **72**, 887–896 (2012).
73. Qin, S. et al. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. *Mol. Cancer* **18**, 155 (2019).
74. Quandt, D., Fiedler, E., Boettcher, D., Marsch, W. & Seliger, B. B7-H4 expression in human melanoma: its association with patients' survival and antitumor immune response. *Clin. Cancer Res.* **17**, 3100–3111 (2011).
75. Kuklinski, L. F. et al. VISTA expression on tumor-infiltrating inflammatory cells in primary cutaneous melanoma correlates with poor disease-specific survival. *Cancer Immunol. Immunother.* **67**, 1113–1121 (2018).
76. Young, A. et al. Targeting adenosine in BRAF-mutant melanoma reduces tumor growth and metastasis. *Cancer Res.* **77**, 4684–4696 (2017).
77. Lee, Y. H. et al. Inhibition of the B7-H3 immune checkpoint limits tumor growth by enhancing cytotoxic lymphocyte function. *Cell Res.* **27**, 1034–1045 (2017).
78. Lee, H. et al. Targeting NK cells to enhance melanoma response to immunotherapies. *Cancers (Basel)* https://doi.org/10.3390/cancers13061363 (2021).
79. Zhou, P. et al. In vivo discovery of immunotherapy targets in the tumour microenvironment. *Nature* **506**, 52–57 (2014).

80. Shifrut, E. et al. Genome-wide CRISPR screens in primary human T cells reveal key regulators of immune function. *Cell* **175**, 1958–1971.e15 (2018).
81. Cabrita, R. et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature* **577**, 561–565 (2020).
82. Helmink, B. A. et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* **577**, 549–555 (2020).
83. Ferrari de Andrade, L. et al. Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell-driven tumor immunity. *Science* **359**, 1537–1542 (2018).
84. He, W. et al. Naturally activated Vγ4 γδ T cells play a protective role in tumor immunity through expression of eomesodermin. *J. Immunol.* **185**, 126–133 (2010).
85. Exley, M. A. et al. Adoptive transfer of invariant NKT cells as immunotherapy for advanced melanoma: a phase I clinical trial. *Clin. Cancer Res.* **23**, 3510–3519 (2017).
86. Petley, E. V. et al. MAIT cells regulate NK cell-mediated tumor immunity. *Nat. Commun.* **12**, 4746 (2021).
87. Yan, J. et al. MAIT cells promote tumor initiation, growth, and metastases via tumor MR1. *Cancer Discov.* **10**, 124–141 (2020).
88. Miracco, C. et al. Utility of tumour-infiltrating $CD25^{+}FOXP3^{+}$ regulatory T cell evaluation in predicting local recurrence in vertical growth phase cutaneous melanoma. *Oncol. Rep.* **18**, 1115–1122 (2007).
89. Gambichler, T., Bindsteiner, M., Hoxtermann, S., Terras, S. & Kreuter, A. Circulating $CD4^{+}CD25^{high}$ $CD127^{low}$ regulatory T cells are an independent predictor of advanced melanoma. *Pigment. Cell Melanoma Res.* **26**, 280–283 (2013).
90. Halaby, M. J. et al. GCN2 drives macrophage and MDSC function and immunosuppression in the tumor microenvironment. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.aax8189 (2019).
91. Ziani, L. et al. Melanoma-associated fibroblasts decrease tumor cell susceptibility to NK cell-mediated killing through matrix-metalloproteinases secretion. *Oncotarget* **8**, 19780–19794 (2017).
92. Huse, M. Mechanical forces in the immune system. *Nat. Rev. Immunol.* **17**, 679–690 (2017).
93. Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J. & Werb, Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat. Commun.* **11**, 5120 (2020).
94. Kaur, A. et al. Remodeling of the collagen matrix in aging skin promotes melanoma metastasis and affects immune cell motility. *Cancer Discov.* **9**, 64–81 (2019).
95. Feng, M. et al. Phagocytosis checkpoints as new targets for cancer immunotherapy. *Nat. Rev. Cancer* **19**, 568–586 (2019).
96. Chang, C. H. et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* **153**, 1239–1251 (2013).
97. Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029–1033 (2009).
98. Swamy, M. et al. Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. *Nat. Immunol.* **17**, 712–720 (2016).
99. Munn, D. H. et al. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J. Exp. Med.* **189**, 1363–1372 (1999).
100. Munn, D. H. et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* **22**, 633–642 (2005).
101. Sundrud, M. S. et al. Halofuginone inhibits T_H17 cell differentiation by activating the amino acid starvation response. *Science* **324**, 1334–1338 (2009).
102. Husain, Z., Huang, Y., Seth, P. & Sukhatme, V. P. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J. Immunol.* **191**, 1486–1495 (2013).
103. Brand, A. et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab.* **24**, 657–671 (2016).
104. Chang, C. H. et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* **162**, 1229–1241 (2015).
105. Stearn, E. W., Sturdivant, B. F. & Stearn, A. E. The life history of a micro-parasite isolated from carcinomatous growths. *Proc. Natl Acad. Sci. USA* **11**, 662–669 (1925).
106. Nejman, D. et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* **368**, 973–980 (2020).
107. Schwabe, R. F. & Jobin, C. The microbiome and cancer. *Nat. Rev. Cancer* **13**, 800–812 (2013).
108. Mehta, R. S. et al. Association of dietary patterns with risk of colorectal cancer subtypes classified by fusobacterium nucleatum in tumor tissue. *JAMA Oncol.* **3**, 921–927 (2017).
109. Garrett, W. S. Cancer and the microbiota. *Science* **348**, 80–86 (2015).
110. Geller, L. T. et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* **357**, 1156–1160 (2017).
111. Yu, T. et al. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* **170**, 548–563.e16 (2017).
112. Chaput, N. et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann. Oncol.* **30**, 2012 (2019).
113. Frankel, A. E. et al. Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. *Neoplasia* **19**, 848–855 (2017).
114. Gopalakrishnan, V. et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97–103 (2018).
115. Matson, V. et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **359**, 104–108 (2018).
116. Routy, B. et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91–97 (2018).
Together with Gopalakrishnan et al. (2018) and Matson et al. (2018), this paper demonstrates how the diversity and composition of gut microbiome can influence the response to ICIs in melanoma and epithelial tumours. The papers also present mouse models for faecal microbiota transplant.
117. Sivan, A. et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084–1089 (2015).
118. Vetizou, M. et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079–1084 (2015).
119. Baruch, E. N. et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* **371**, 602–609 (2021).
120. Davar, D. et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* **371**, 595–602 (2021).
Together with Baruch et al. (2021), this paper demonstrates clinical benefits to patients with melanoma of faecal microbiota transplantation to overcome ICI resistance.
121. Uribe-Herranz, M. et al. Gut microbiota modulates adoptive cell therapy via CD8α dendritic cells and IL-12. *JCI Insight* https://doi.org/10.1172/jci.insight.94952 (2018).
122. Tanoue, T. et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* **565**, 600–605 (2019).
123. Poore, G. D. et al. Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature* **579**, 567–574 (2020).
Together with Nejman et al. (2020) and Geller et al. (2017), this paper demonstrates the presence of bacteria in human tumour samples using experimental or computational methods. Geller et al. (2017) also present a bacteria-driven resistance mechanism to chemotherapy.
124. Zhu, G. et al. Intratumour microbiome associated with the infiltration of cytotoxic $CD8^{+}$ T cells and patient survival in cutaneous melanoma. *Eur. J. Cancer* **151**, 25–34 (2021).
125. Pushalkar, S. et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov.* **8**, 403–416 (2018).
126. Gur, C. et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* **42**, 344–355 (2015).
This study presents mechanisms by which *F. nucleatum* evades the immune response in the melanoma TME.
127. Hamada, T. et al. *Fusobacterium nucleatum* in colorectal cancer relates to immune response differentially by tumor microsatellite instability status. *Cancer Immunol. Res.* **6**, 1327–1336 (2018).
128. Thomas, N. E. et al. Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study. *J. Clin. Oncol.* **31**, 4252–4259 (2013).
129. Chen, P. L. et al. Analysis of immune signatures in longitudinal tumor samples yields insight into biomarkers of response and mechanisms of resistance to immune checkpoint blockade. *Cancer Discov.* **6**, 827–837 (2016).
130. Tumeh, P. C. et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
131. Riaz, N. et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* **171**, 934–949.e16 (2017).
132. Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G. & Hacohen, N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* **160**, 48–61 (2015).
133. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **175**, 998–1013. e20 (2018).
134. Yost, K. E. et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat. Med.* **25**, 1251–1259 (2019).
135. Topalian, S. L. et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* **366**, 2443–2454 (2012).
136. Wolchok, J. D. et al. Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **369**, 122–133 (2013).
137. Robert, C. et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* **372**, 320–330 (2015).
138. Grasso, C. S. et al. Conserved interferon-γ signaling drives clinical response to immune checkpoint blockade therapy in melanoma. *Cancer Cell* **38**, 500–515.e3 (2020).
139. Ayers, M. et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J. Clin. Inves.* **127**, 2930–2940 (2017).
140. Snyder, A. et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014).
141. Spranger, S. et al. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. *Proc. Natl Acad. Sci. USA* **113**, E7759–E7768 (2016).
142. Hugo, W. et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* **165**, 35–44 (2016).
143. Ribas, A. et al. Combined BRAF and MEK inhibition with PD-1 blockade immunotherapy in BRAF-mutant melanoma. *Nat. Med.* **25**, 1319–1319 (2019).
144. Ascierto, P. A. et al. Dabrafenib, trametinib and pembrolizumab or placebo in BRAF-mutant melanoma. *Nat. Med.* **25**, 941–946 (2019).
145. Sullivan, R. J. et al. Atezolizumab plus cobimetinib and vemurafenib in BRAF-mutated melanoma patients. *Nat. Med.* **25**, 929–935 (2019).
146. Ribas, A. et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. *Cell* **170**, 1109–1119 (2017).
147. Singh, M. et al. Intratumoral CD40 activation and checkpoint blockade induces T cell-mediated eradication of melanoma in the brain. *Nat. Commun.* https://doi.org/10.1038/s41467-017-01572-7 (2017).
148. Sahin, U. et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* **585**, 107–112 (2020).
Together with Sahin et al. (2017), Ott et al. (2017) and Hu et al. (2021), this paper describes the use of peptide and RNA vaccines with mutated antigens or tumour-associated antigens in patients with melanoma. The success of these studies demonstrates the power of selecting the right antigens and using them for vaccines.
149. Apetoh, L. et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.* **13**, 1050–1059 (2007).
150. Twyman-Saint Victor, C. et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* **520**, 373–377 (2015).
151. Morgan, R. A. et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* **314**, 126–129 (2006).
152. Stadtmauer, E. A. et al. CRISPR-engineered T cells in patients with refractory cancer. *Science* https://doi.org/10.1126/science.aba7365 (2020).

153. Wargo, J. A. Modulating gut microbes. *Science* **369**, 1302–1303 (2020).
154. Harlin, H. et al. Chemokine expression in melanoma metastases associated with CD8$^+$ T-cell recruitment. *Cancer Res.* **69**, 3077–3085 (2009).
155. Rosenberg, S. A. IL-2: the first effective immunotherapy for human cancer. *J. Immunol.* **192**, 5451–5458 (2014).
156. Zhang, S. et al. Systemic interferon-γ increases MHC class I expression and T-cell infiltration in cold tumors: results of a phase 0 clinical trial. *Cancer Immunol. Res.* **7**, 1237–1243 (2019).
157. Sun, Z. et al. A next-generation tumor-targeting IL-2 preferentially promotes tumor-infiltrating CD8$^+$ T-cell response and effective tumor control. *Nat. Commun.* **10**, 3874 (2019).
158. Ribas, A. et al. SD-101 in combination with pembrolizumab in advanced melanoma: results of a phase Ib, multicenter study. *Cancer Discov.* **8**, 1250–1257 (2018).
159. Andre, P. et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. *Cell* **175**, 1731–1743.e13 (2018).
160. Godfrey, D. I., Le Nours, J., Andrews, D. M., Uldrich, A. P. & Rossjohn, J. Unconventional T cell targets for cancer immunotherapy. *Immunity* **48**, 453–473 (2018).
161. Klebanoff, C. A., Rosenberg, S. A. & Restifo, N. P. Prospects for gene-engineered T cell immunotherapy for solid cancers. *Nat. Med.* **22**, 26–36 (2016).
162. Liu, E. L. et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N. Engl. J. Med.* **382**, 545–553 (2020).
163. Klichinsky, M. et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat. Biotechnol.* **38**, 947–953 (2020).
164. Cho, J. H. et al. Engineering advanced logic and distributed computing in human CAR immune cells. *Nat. Commun.* https://doi.org/10.1038/s41467-021-21078-7 (2021).
165. Kalaora, S. et al. Use of HLA peptidomics and whole exome sequencing to identify human immunogenic neo-antigens. *Oncotarget* **7**, 5110–5117 (2016).
166. Bassani-Sternberg, M. et al. Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry. *Nat. Commun.* **7**, 13404 (2016).
167. Pritchard, A. L. et al. Exploration of peptides bound to MHC class I molecules in melanoma. *Pigment. Cell Melanoma Res.* **28**, 281–294 (2015).
168. Robbins, P. F. et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat. Med.* **19**, 747–752 (2013).
169. Gros, A. et al. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. *Nat. Med.* **22**, 433–438 (2016).
170. Prickett, T. D. et al. Durable complete response from metastatic melanoma after transfer of autologous T cells recognizing 10 mutated tumor antigens. *Cancer Immunol. Res.* **4**, 669–678 (2016).
171. Lennerz, V. et al. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. *Proc. Natl Acad. Sci. USA* **102**, 16013–16018 (2005).
172. Linnemann, C. et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4$^+$ T cells in human melanoma. *Nat. Med.* **21**, 81–85 (2015).
173. Andersen, R. S. et al. Dissection of T-cell antigen specificity in human melanoma. *Cancer Res.* **72**, 1642–1650 (2012).
174. Kvistborg, P. et al. TIL therapy broadens the tumor-reactive CD8$^+$ T cell compartment in melanoma patients. *Oncoimmunology* **1**, 409–418 (2012).
175. Cohen, C. J. et al. Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes. *J. Clin. Invest.* **125**, 3981–3991 (2015).
176. The Human Microbiome Project Consortium. A framework for human microbiome research. *Nature* **486**, 215–221 (2012).
177. DeSantis, T. Z. et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Env. Microbiol.* **72**, 5069–5072 (2006).
178. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2013).
179. Cole, J. R. et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **42**, D633–D642 (2014).
180. Zhang, J. et al. Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Sci. Total. Env.* **618**, 1254–1267 (2018).
181. Bashiardes, S., Zilberman-Schapira, G. & Elinav, E. Use of metatranscriptomics in microbiome research. *Bioinform Biol. Insights* **10**, 19–25 (2016).
182. Seng, P. et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin. Infect. Dis.* **49**, 543–551 (2009).
183. Clark, A. E., Kaleta, E. J., Arora, A. & Wolk, D. M. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. *Clin. Microbiol. Rev.* **26**, 547–603 (2013).

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