De-novo and acquired resistance to immune checkpoint targeting

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Use of immune checkpoint inhibitors targeting the programmed cell death protein-1/programmed cell death-ligand 1 and cytotoxic T lymphocyte-associated protein-4 axes has yielded impressive results in some clinical trials. However, only a subset of patients initially respond to these inhibitors, and increasing clinical evidence indicates that a substantial proportion of initial responders ultimately relapse with lethal, drug-resistant disease months or years later. Studies that have used massively parallel sequencing have shed light on the rich functional landscape of mutations that endow tumour cells with the ability to evade T-cell-mediated immunosurveillance. Cancer genomes bear signatures of clonal evolution and selection, particularly implicating acquired defects in interferon receptor signalling and antigen presentation. In this Review, we discuss the biological processes that operate in the formation of so-called immunoresistant niches, and describe the latest progress in the development of combination strategies to reinstate immunosurveillance in immune-refractory tumours.

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Introduction

Since the beginning of the 20th century, when Paul Ehrlich[1] formulated his enduring immune surveillance hypothesis, which proposed the immune system was centrally important for eradication of the overwhelming frequency of clinically undetectable carcinomas, research on cancer and immunity has focused on how a subpopulation of malignant cells eventually escape immunological control to establish macroscopic and clinically manifesting colonies. A direct consequence of these conceptual foundations was the premise that effective cancer treatment hinges on our capacity to block—or even reverse—immune-escape mechanisms. This field has been validated by seminal work from Leach and colleagues,[2] who showed that blockade of T-cell suppressive pathways could unchain T-cell-dependent rejection of pre-established cancers in immunocompetent mouse models.

Immune checkpoints are orchestrated by a set of costimulatory and inhibitory molecules, which regulate the activation and effector functions of T lymphocytes. These regulatory circuits enable self-tolerance under normal physiological contexts but frequently become coopted in malignancy. Accordingly, immune checkpoint blockers—such as ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]), pembrolizumab and nivolumab (anti-programmed cell death protein-1 [PD-1]), and atezolizumab, durvalumab, and avelumab (anti-programmed cell death ligand-1 [PD-L1])—have shown activity in clinical trials, and are gaining approval for an expanding array of indications. These indications include metastatic melanoma,[3–10] advanced non-small-cell lung cancer,[11–14] renal cell carcinoma,[15] classic Hodgkin's lymphoma,[16–19] urothelial cancers,[20–23] squamous cell cancer of the head and neck,[24–27] Merkel cell carcinoma,[28,29] and, more recently, solid tumours that show microsatellite instability (MSI-H) and mismatch repair deficiency.[25,30–33]

Despite the transformative potential of immune checkpoint blockers, upfront clinical benefits in approved indications are not universal. For patients with either metastatic melanoma or non-small-cell lung cancer for instance, 19–45% of unselected, previously treated patients,[3–5,14] or 40–45% of patients with PD-L1-positive tumours in the frontline setting, achieved an objective response to anti-PD-1 monotherapy.[4,11] The combination of nivolumab plus ipilimumab in previously untreated patients with metastatic melanoma yielded a response rate of 72% among patients who were PD-L1-positive and 55% among patients who were PD-L1-negative.[4] However, the prospect of broad therapeutic efficacy of immune checkpoint blockers across multiple tumour histologies remains elusive, such as in the treatment of pancreatic ductal adenocarcinoma and metastatic castration-resistant prostate cancer, which are largely resistant to checkpoint targeting approaches.

Although early murine studies[2] encouraged the notion that immune checkpoint blockers can engender long-lived protection against neoplasms, clinical follow-up results have disputed these expectations. For example, in the EORTC 18071 trial,[6,7] more than half of high-risk patients with stage III melanoma randomised to receive adjuvant ipilimumab had disease relapse, with a median recurrence-free survival of 26·1 months. In the KEYNOTE-001 trial,[3] about one in four patients with metastatic melanoma who achieved an initial objective response to pembrolizumab subsequently had disease progression during follow-up (median 21 months). These results indicate that a substantial proportion of patients treated with immune checkpoint inhibitors might eventually acquire therapeutic resistance.

Enumerating the underlying mechanisms of de-novo (or primary) and acquired resistance to immune checkpoint targeting strategies has thus become a logical next step for cancer research. In this Review, we set out an organising framework (figure 1) for understanding immune-escape mechanisms in these contexts. In addition, we highlight emerging treatment approaches that might prolong the efficacy of immune checkpoint

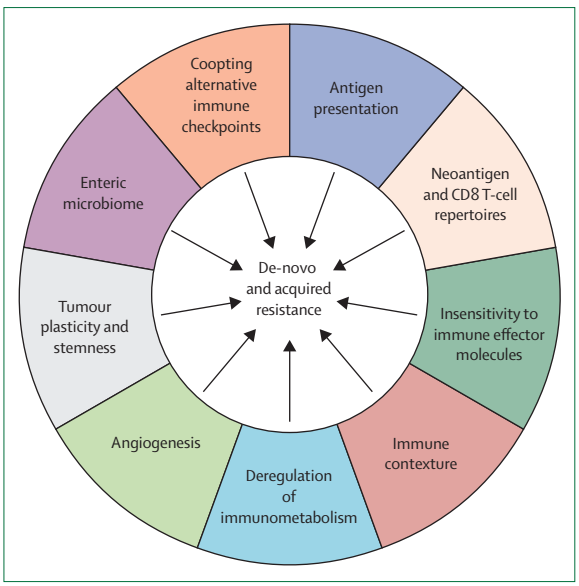

***Figure 1*: Mechanisms operating in the establishment of immunoresistant niches**

blockers or enable immunotherapy to impinge on previously intractable cancer types.

Two sides of the same coin?

Research during the past decade has identified a myriad of mechanisms that cause primary resistance to immune checkpoint blockade. By contrast, until recently, very little was understood about the acquired resistance pathways that underlie delayed relapses in patients who have received immunotherapy. Nevertheless, substantial parallels exist in the mechanisms of tumour escape for both primary and acquired resistance, suggesting a unifying conceptual framework that could provide greater integrative understanding of these two mechanisms. However, it is important to appreciate the contextual nuances in the basis of these two phenotypes, which will hopefully be largely explained in this Review. Crucially, although most of our insights into immune escape processes concentrate on cancer cell autonomous processes, it is increasingly clear that the heterotypic, ostensibly normal cell types that exist in the tumour microenvironment might also play a part in the formation of spatially limited immunoresistant niches (figure 1). In this Review, we illustrate these mechanistic insights using data from the past decade, which might form the basis to future therapeutic efforts against immunotherapy-refractory cancers.

Defective tumour immunorecognition

Recognition of cancer cells by the adaptive immune system is the most important requirement for tumour rejection, and encompasses the individual steps of tumour antigen presentation and priming of naive T cells. This multistep process appears to be deregulated in many immunotherapy-resistant tumours (figure 2).

Antigen presentation

Cancers express a wide range of MHC peptides, which can be targets for specific cytotoxic T lymphocytes (CTLs), causing them to become immunogenic. Consistent with this notion, carcinomas with robust T cell immunosurveillance—either natural or therapy-induced—have been shown to disable antigen presentation to evade immunorecognition through genetic and epigenetic means (figure 2).[34–40] In the context of natural immunosurveillance (not induced by therapies), mutations in the antigen processing and presentation pathways are substantially enriched in multiple tumour types with robust T-cell infiltration or immune cytolytic activity.[34,35] Giannakis and colleagues[34] reported that most of the human leucocyte antigen mutations in highly-infiltrated colorectal tumours recurred in the exon 4 region, which corresponds to the T cell receptor binding domain, indicating that they were likely to have been clonally selected for their ability to impair T cell immunorecognition.

Multiple lines of evidence have delineated a crucial role for genetic deficiencies of β-2-microglobulin (B2M)—the invariant chain of MHC, which is essential for proper MHC class I folding and transport to the cell surface—in promotion of de-novo and acquired resistance to immunotherapies.[34–38] Zaretsky and colleagues[37] showed that acquired resistance to PD-1 blockade was accompanied by acquisition of a new 4-bp homozygous frameshift deletion in *B2M* in a late-relapse patient with metastatic melanoma, who had a progression-free survival of 14·9 months despite continuous dosing. Furthermore, loss of B2M has been associated with de-novo resistance to CTLA-4 blockade.[41] These findings validate the notion that a proficient tumour antigen presentation pathway is required for a successful response to immunotherapy. Nevertheless, antigen-loss variants could be eradicated indirectly via bystander elimination when tumour-specific CTLs in the vicinity destroy stromal myeloid cells that cross-present tumour epitopes, or by MHC class II-mediated CD4 T-cell immunorecognition of tumour antigens, representing two potential therapeutic avenues that warrant further investigation.[42]

Neoantigen repertoire

The cancer immunoediting hypothesis posits that frequent interactions between the immune system and cancer cells during the life history of a tumour eventually settle in a so-called evolutionary cul-de-sac, in which the immune system can no longer recognise the tumour.[43] Indeed, the molecular footprints of T-cell-dependent immunoediting (ie, the depletion of antigen-generating point mutations that are required for immunorecognition and productive antitumour

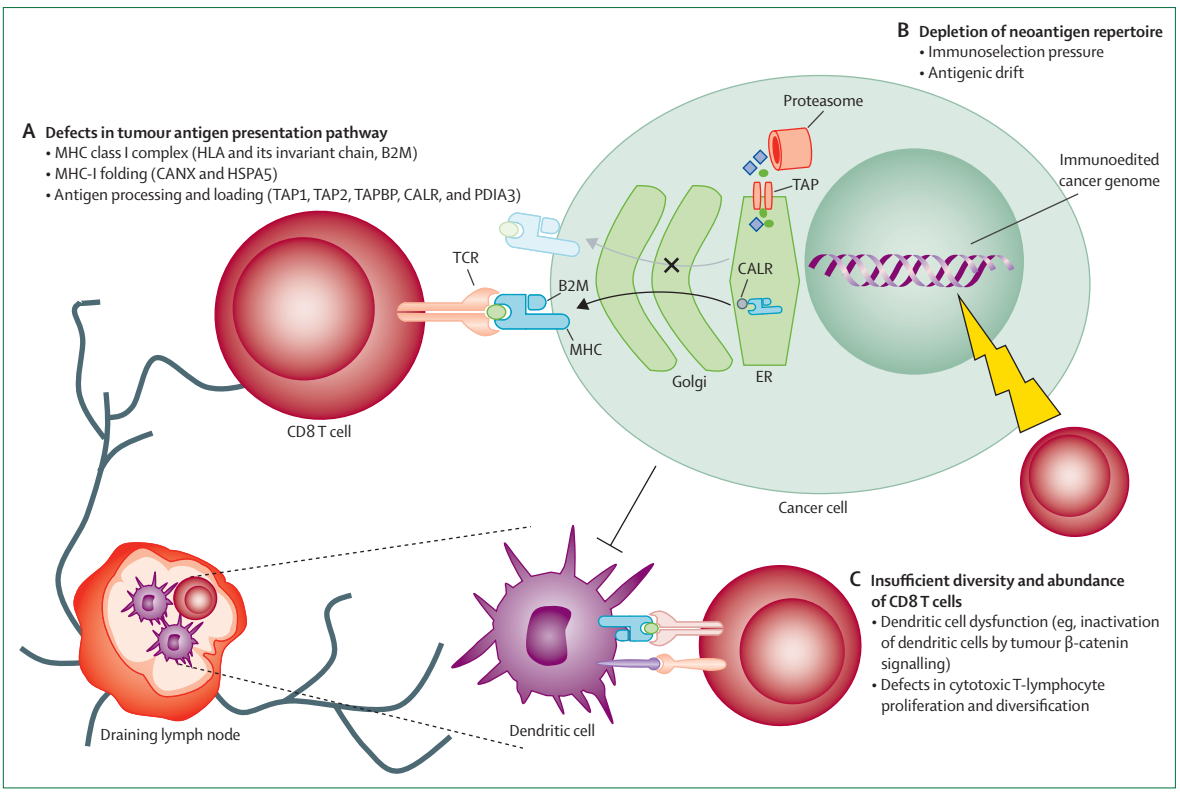

***Figure 2*: Impaired immunorecognition of tumour cells during checkpoint blockade immunotherapy**
(A) Defects in the tumour antigen processing and presentation machinery can render a tumour cell invisible to tumour-specific CD8 T cells. (B) During a therapy-elicited immune response, cancer cells that express specific peptide MHCs are selectively culled by antigen-specific T cells. Interferon-γ released by immune effector cells might expedite this immunoediting process. As a result, tumour clones emerge that do not bear the original cognate antigens required for productive immune recognition. (C) Decreased abundance and diversity of intratumoural activated CD8 T cells, which might result from deregulation of naive T-cell priming and proliferation, can allow tumours to escape anticancer immunosurveillance. HLA=human leucocyte antigen. B2M=β-2-microglobulin. CANX=calnexin. HSPA5=heat shock 70 kDa protein 5. TAP=transporter associated with antigen processing. TAPBP=transporter associated with antigen processing binding protein. CALR=calreticulin. PDIA3=protein disulfide-isomerase A3. TCR=T-cell receptor. ER=endoplasmic reticulum.

responses during immunotherapy) are found prominently inscribed in the genomes of established cancers.[35,44,45]

Evidence has emerged[46,47] that immunoediting might promote acquired resistance to immune checkpoint blockers (figure 2). Anagnostou and colleagues[46] showed that relapsed tumours lost 7–18 putative neoantigens each by comparing the neoantigen landscape of matched pretreatment and relapsed biopsies from patients with non-small-cell lung cancer treated with anti-PD-1 or an anti-PD-1 plus anti-CTLA-4 combination. The lost neoantigens had higher predicted affinities for autologous *MHC* variants and elicited stronger T-cell receptor responses in peripheral blood lymphocytes compared with neoantigens that were retained or gained in the relapsed tumour, indicating that the selective depletion of these antigens might constitute bona-fide immunoediting.[46] In line with these observations, a 2017 study[47] unmasked a paradoxical role for interferon-γ (the production of which is upregulated during T-cell-based immunotherapy and assists in tumour killing) in expediting CTL-dependent cancer genome immunoediting. In future studies, it might be interesting to inspect the cancer genomes of primary-resistant tumours for enrichment of molecular signatures from immunoediting.

CD8 T-cell repertoire

The abundance and diversity of tumour-specific CTLs are pivotal elements to consider when assessing a tumour's visibility to the immune system. Failure of immune cells to launch subsequent waves of attack to contain emergent cancer clones (showing an evolved complement of neoantigens),[46] or the fact that adoptive transfer of tumour-infiltrating lymphocytes can mediate a high rate of objective responses from patients with metastatic melanoma who have progressed on anti-CTLA-4/anti-PD-1 blockade,[48] seems to ascribe immune checkpoint blocker resistance to insufficient breadth (ie, monoclonality) or depth of T-cell responses. Therefore, mechanisms leading to the inability of T cells to proliferate and diversify,[41,49,50] which could occur at the level of naive T-cell priming,[50] could potentially contribute to the preparation of immunoresistant niches (figure 2). This process was perhaps elegantly shown in a study[50]

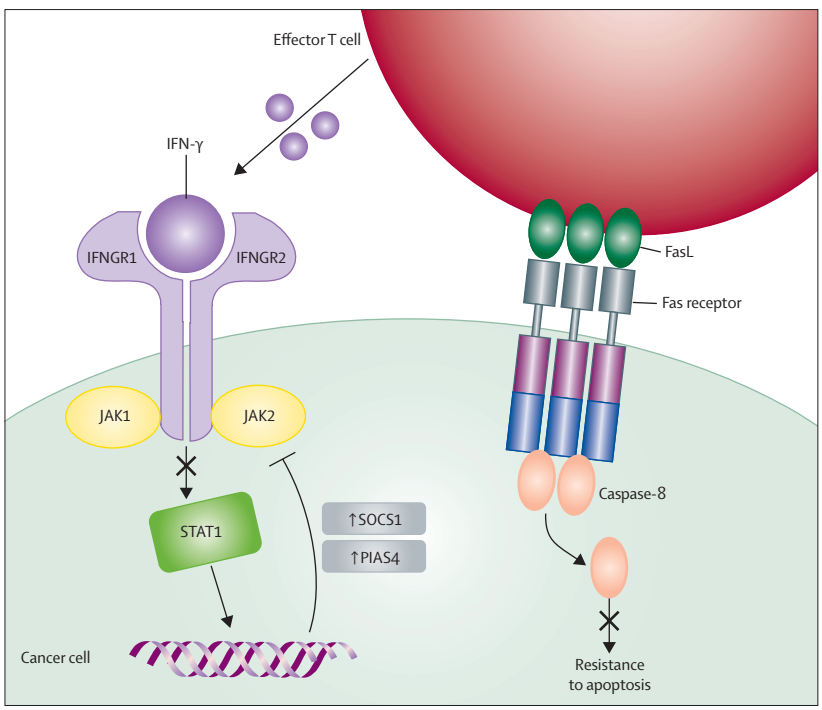

***Figure 3*: Insensitivity to T-cell effector molecules**
Tumour cells can become resistant to the antiproliferative and proapoptotic effects of T-cell effector molecules through mutational or epigenetic inactivation, and enhanced negative feedback regulation of signal transduction pathways. IFN=interferon. IFNGR=interferon-γ receptor. JAK=Janus kinase. STAT=signal transducer and activator of transcription. SOCS1=suppressor of cytokine signalling 1. PIAS4=protein inhibitor of activated STAT4. Fas=apoptosis antigen 1. FasL=ligand for FAS receptor.

that found that suppression of CD103 dendritic-cell recruitment by tumour-intrinsic active β-catenin signalling interceded intratumoural T-cell exclusion, which led to primary resistance to anti-PD-L1/anti-CTLA-4 blockade in a mouse melanoma model. In another study,[51] an absence of Batf3-lineage dendritic cells in melanoma was found to impair the priming and recruitment of T cells, resulting in a non-T-cell inflamed state. Potential therapeutic strategies to salvage this form of immunotherapy-resistant tumours include use of cancer vaccines to augment the T-cell response and generate de-novo T cells, and use of radiation to enhance the diversity of the intratumoural repertoire of T-cell receptors.[52]

Insensitivity to immune effector molecules

Tumour destruction is classically conceived to be principally affected through perforin-mediated and granzyme-mediated lysis. However, T-cell effector cytokines, such as interferon-γ, directly help to restrain tumour growth by exerting direct antiproliferative and proapoptotic effects on cancer cells, and indirectly help via upregulation of tumour antigen presentation machinery (eg, inducible proteasome subunits, transporter associated with antigen processing 1/2, and MHC complex).

Interferon-γ mainly uses the Janus kinase (JAK) signal transducer and activator of the transcription pathway to activate *STAT1*, which mediates the immune effector functions of interferon-γ. Loss of interferon-γ signalling can therefore render tumour cells less susceptible to attacking T cells and thereby mediate resistance to immune checkpoint blockers (figure 3). Two recent studies[37,53] have implicated inactivating mutations in *JAK1* and *JAK2* in the mediation of clinical primary and acquired resistance to PD-1 blockade. In-vitro experiments showed that these mutations led to the loss of interferon-γ-induced expression of MHC class I and PD-L1, phosphorylation of the STAT1 transcription factor, and interferon-γ-mediated growth arrest.[37,53] In another study,[54] around 75% of patients who did not respond to CTLA-4 blockade harboured somatic genomic defects in the interferon-γ pathway (eg, interferon-γ receptor [*IFNGR*]*1*, interferon regulatory factor 1, *JAK2*, and *IFNGR2*) and amplification of major interferon-γ pathway negative regulators (eg, suppressor of cytokine signalling 1 and protein inhibitor of activated STAT4). Apart from mutational defects, STAT1-related epigenomic aberrations induced by persistent type II interferon signalling have also been shown to disrupt interferon-γ signal transduction and mediate resistance to anti-PD-1 and anti-CTLA-4 blockers.[55] Potential therapeutic strategies for targeting JAK2 loss include use of stimulator of interferon genes agonists, which can activate STAT1 in a JAK2-independent fashion, or activation of the type I interferon pathway.[37,56,57] Furthermore, oncolytic viruses (eg, vesicular stomatitis virus and Newcastle disease virus) that show a proclivity for replication in neoplastic cells with defective interferon signalling could, in the future, be exploited against tumours that have acquired resistance to effector T-cell molecules (table).[58,59] Additionally, oncolytic therapy has been successfully implemented in the clinic with the approval of talimogene laherparepvec in advanced, unresectable melanoma, and H101 adenovirus in advanced, refractory nasopharyngeal cancer.[60]

Additional mechanisms that allow cancers to resist T-cell effector molecules might exist and warrant further investigation. For instance, caspase 8 (*CASP8*), a key part of the extrinsic apoptosis pathway, was found to be the most recurrently mutated gene associated with immune cytolytic activity, especially in immunogenic cancers such as head and neck cancer, colorectal cancer, lung squamous cell carcinoma, and uterine cancer.[35] In this study, the diffuse pattern of mutations suggested loss-of-function, implying that recurrent *CASP8* mutations could potentially prevent CTLs from killing tumours via apoptosis antigen 1 ligand (FasL)/Fas interactions. However, to date, no direct evidence exists to show that inactivating *CASP8* mutations can mediate immunotherapeutic resistance.

Tumour microenvironment and neovasculature

The immunoresistant niche is formed by cancer cells and other components of the tumour ecosystem (figure 4).

	Potential therapeutic avenues	Selected ongoing clinical trials
Immunorecognition: defective antigen presentation in tumour cells; immunoediting; limited tumour-specific cytotoxic T lymphocyte diversity and abundance	Cancer vaccines; radiotherapy and cytotoxic therapy; targeting stromal myeloid cells in tumour microenvironment or harnessing MHC class II-mediated T-cell immunorecognition; epigenetic therapies to induce neoantigen re-expression	NCT02635360; NCT02880345; NCT03040999; NCT02648282; NCT02437136; NCT02664181; NCT02701400
Tumour insensitivity to T cell effector molecules	Stimulator of interferon genes agonists; oncolytic viruses that replicate in cells with defective interferon signalling (eg, vesicular stomatitis virus and Newcastle disease virus)	NCT02675439; NCT03172936; NCT02923466; NCT01628640; NCT03120624
Immunosuppressive tumour microenvironment and neovasculature: T-cell exhaustion and exclusion; deregulation of immunometabolism; aberrant angiogenesis and vasculogenesis	Epigenetic therapies (eg, DNA methyltransferase 1 inhibitor, histone deacetylase inhibitor); modulation of immune cell subsets and chemokine profiles (eg, COX inhibitors, celecoxib); targeted inhibition of phosphoinositide 3-kinase γ in myeloid cells; class IIa histone deacetylase inhibition in monocytes and macrophages; targeted therapies that neutralise myeloid-derived suppressor cell functions (eg, cabozantinib, BEZ235); targeting immunometabolism: modulation of glycolysis, inhibition of adenosinergic and kynurenine pathways; targeting the neovasculature: VEGF inhibitors (eg, axitinib, sunitinib, and bevacizumab)	NCT02637531; NCT02853331; NCT02452424; NCT02437136; NCT02664181; NCT02655822; NCT03024437; NCT02395627; NCT02014636; NCT02268825; NCT03026140; NCT03141177; NCT03149822; NCT02856425; NCT02788279
Tumour plasticity and stemness	Inhibition of tumour necrosis factor signalling; epithelial-to-mesenchymal transition inhibitors	NCT02690948; NCT02872259; NCT03184571; NCT03184558; NCT02722954
Enteric microbiome	Antibiotics that decrease Gram-positive bacteria but not the Gram-negative Bacteroidales and Burkholderiales families (eg, vancomycin); probiotics supplementation (eg, *Bifidobacterium* spp, including *Bifidobacterium breve* and *Bifidobacterium longum*)	..
Compensatory upregulation of multiple inhibitory and costimulatory immune checkpoints	Combination therapy involving selective inhibitors against T-cell modulatory axes including lymphocyte-activation gene-3, T-cell immunoreceptor with immunoglobulin and ITIM domains, T-cell immunoglobulin and mucin-domain containing-3, and V-domain immunoglobulin suppressor of T-cell activation	NCT02327078; NCT02655822; NCT02073123; NCT02460224; NCT01604889; NCT02794571; NCT02913313; NCT01968109; NCT02658981; NCT02720068

***Table*: Selected studies exploring immunoresistance mechanisms**

Elucidation of an innate immune checkpoint blocker resistance-related transcriptional signature, which is associated with many cancer cell non-autonomous pathways, including angiogenesis, extracellular matrix remodelling, and wound healing,[61] seems to support this point.

Immune contexture

Although some tumours have a smouldering yet ineffective inflammatory response, others show few signs of lymphocytic infiltration. These gradations in T-cell infiltration[37,50] and reactivity[62–66] have clinical implications for patients treated with immunotherapy, and studies have sought to elucidate their mechanistic bases in greater detail (figure 4). In the context of acquired resistance to PD-1 blockade, Zaretsky and colleagues[37] showed that biopsies obtained at the time of response had a marked increase in intratumoural CD8 T-cell infiltrate density, whereas relapsed lesions showed a reversal back to a T cell excluded state (wherein CD8 T cells are largely restricted to the invasive margin). This finding is important because it reinforces the idea that converging mechanisms might underlie de-novo and acquired immunotherapy resistance. Furthermore, this finding validates the notion that dynamic changes in T-cell infiltrates at the tumour centre correlate with the onset of resistance, which might open new possibilities for predictive applications. Treatments that prime intratumoural T-cell infiltration, such as the use of chemotherapy drugs (eg, cyclophosphamide and oxaliplatin) or targeted therapies that act as immune adjuvants, might therefore represent promising approaches for the delay or reversal of immune checkpoint blocker resistance.[67,68]

Fresh insights into the epigenetic basis of T-cell exhaustion and its association with primary immune checkpoint blocker resistance[62–65] have also exposed novel targets for therapy. A growing body of findings suggests that hardwired epigenomic modifications might underlie CD8 T-cell exhaustion. Anti-PD-1 or anti-PD-L1 treatment partly rescues cytokine production by dysfunctional tumour-infiltrating lymphocytes, but does not elicit anergic CD8 T cells to acquire a memory T-cell phenotype, thus limiting the durability of immune checkpoint inhibition.[62–65] These findings suggest that epigenetic therapies, such as histone deacetylase inhibitors, or combination strategies that simultaneously antagonise inhibitory circuitries while activating costimulatory pathways, could prove useful to maximally revitalise exhausted CD8 T cells.[64,65] Hence, the phase 2b ENCORE 601 (NCT02437136) trial and the phase 2 PRECISE trial (NCT02664181) are examples of ongoing studies that are testing the combination of epigenetic therapies (eg, DNA methyltransferase 1 inhibitor and histone deacetylase inhibitor) with PD-1 inhibitors in patients who are refractory to immune checkpoint inhibition, of which preliminary results have suggested favourable disease control.[69]

T-cell infiltration and reactivity are also influenced by chemokine profiles and relative abundances of immune cell subsets (eg, myeloid-derived suppressor cells and tumour-associated macrophages) in the extracellular milieu, the detailed pathomechanisms of which have been reviewed elsewhere.[70,71] Crucially, pharmacological strategies targeting these axes are being actively developed and have yielded relatively exciting preclinical results (table).[72–75]

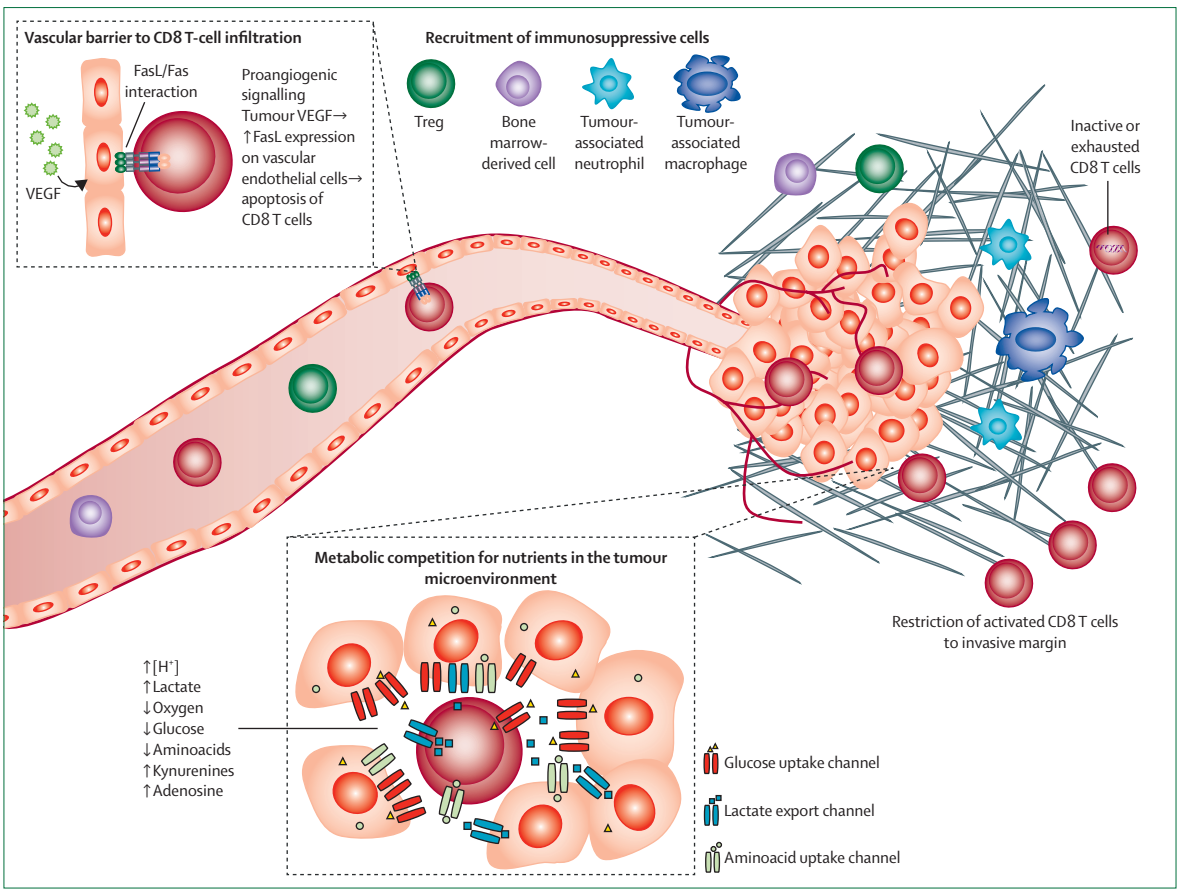

***Figure 4*: Tumour microenvironment and neovasculature**
Tumour endothelium plays a part in engendering spatially-limited immunoresistant niches by expressing the FasL ligand, which kills effector CD8 T cells, and also by posing as a physical barrier to prevent extravasation into the tumour parenchyma. Furthermore, tolerogenic cell types and immunosuppressive molecules present in the tumour microenvironment and metabolic competition for nutrients have been shown to induce exhaustion and inactivation of CD8 T-cell infiltrates, thereby limiting the durability of immune checkpoint inhibition. Fas=apoptosis antigen 1. FasL=ligand for FAS receptor. Treg=regulatory T cell.

Deregulation of immunometabolism

Immune cells undergo complex shifts in metabolic states when they mount an immune response. CD8 T cells, for example, are highly dependent on aerobic glycolysis to fuel their metabolic demands during the effector phase.[76] However, bioenergetic constraints (eg, acute hypoxia, high concentrations of tumour-derived lactate, and glucose and aminoacid deprivation) imposed by the tumour microenvironment can encumber proper T cell activation and effector functions, and are now increasingly recognised to contribute to an abnormal immunosurveillance axis in cancer.[76–78]

Derangements of T-cell immunometabolism might constitute another mechanism of resistance to checkpoint modulatory approaches (figure 4). A high concentration of serum lactate dehydrogenase, for example, is known to correlate with primary resistance and poor outcomes on CTLA-4 and PD-1 blockade.[77,79–83] A database analysis[77] of patients with melanoma revealed strong negative associations between tumour lactate dehydrogenase expression and markers of CTL activation. These findings might be explained by the inability of CD8 T cells to export lactate in the presence of a high extracellular concentration of tumour-derived lactic acid, which blunts aerobic glycolysis.[77,79,80] By contrast, regulatory T cells are not as susceptible to this tumour microenvironment as they use other metabolic pathways such as the catabolism of fatty acids and mitochondrial oxidative phosphorylation. Separately, indoleamine 2,3-dioxygenase, the rate-limiting enzyme in the conversion of tryptophan to kynurenines, also disrupts proliferation and signalling by starving T cells of tryptophan and generating toxic metabolites. In a phase 1 study[84] of pembrolizumab plus the indoleamine 2,3-dioxygenase inhibitor, epacadostat, 14 of 19 patients with advanced melanoma experienced clinical benefit (six achieved a complete response, five achieved a partial response, and three had stable disease).

Angiogenesis

The tumour neovasculature poses a formidable physical barrier to the trafficking and extravasation of

lymphocytes. Upregulation of *VEGF* (which also functions as an immunosuppressive cytokine) and genes involved in proangiogenic signalling is reportedly associated with the development of primary[61,85,86] and acquired resistance[87] to immune checkpoint blockers (figure 4). Proangiogenic VEGF signalling was shown to induce expression of the cell death mediator ligand FasL on vascular endothelial cells, which selectively culls effector CD8 T cells while permitting extravasation of tolerogenic FoxP3-positive regulatory T cells.[88] Crucially, vascular normalisation with anti-angiogenic targeted therapies reverses immunotherapy resistance,[85,89] and is associated with a concomitant increase in lymphocyte trafficking and migration across the endothelium.[85,90] These data indicate that tumour angiogenesis and vasculogenesis are key processes that aid the formation and sustenance of spatially-limited immunoresistant niches. The anti-VEGF plus anti-PD1/PD-L1 or anti-CTLA-4 combination has shown substantial potency, especially in renal cell carcinoma, and has transitioned into phase 3 testing (NCT03141177 and NCT02853331; table).

Tumour plasticity and stemness

Therapy-induced inflammation is known to promote tumour plasticity and phenotypic heterogeneity among cancer cells, which underpins therapeutic resistance to cytotoxic drugs, radiotherapy, and targeted therapies. A pertinent question that arises is whether reactive immune infiltrates might also be responsible, however paradoxically, for promoting resistance to immunotherapies. Indeed, augmented expression of genes (eg, *AXL, TWIST2, WNT5A, LOXL2, ROR2, TAGLN,* and *FAP*) involved in the epithelial-to-mesenchymal transition has been identified in a major subset of primary-resistant tumours that did not respond to PD-1 blockade.[61] A subsequent study[91] determined that this epithelial-to-mesenchymal transition transcriptional signature was associated with tumour necrosis factor α (TNFα), which drove translation reprogramming and culminated in enhanced phenotypic plasticity. Translation reprogramming was found to recapitulate the innate immune checkpoint blocker resistance-related transcriptional signature gene expression programme, and encourage tumour resistance to adoptive T-cell transfer therapy.[91] This finding corroborates earlier studies,[92,93] which also reported that a mesenchymal-like phenotype switch or TNFα-mediated dedifferentiation of melanoma cells might mediate resistance to adoptive T-cell transfer protocols. Notably, blocking TNFα signalling with etanercept enhances CD8 T-cell-dependent immunity in experimental melanoma, possibly by preventing TNF-induced CD8 T-cell death.[93]

In the context of natural immunosurveillance, CD8 T cells have also been shown to induce epithelial-to-mesenchymal transitioning in epithelial breast cancer, and generate cancer stem cells leading to the T-cell-dependent outgrowth of breast tumours in a mouse model.[94] Integrated analyses[95] of several large molecular datasets (TCGA, PROSPECT, and BATTLE-1) also showed that inflammatory changes in the tumour microenvironment were strongly associated with induction of an epithelial-to-mesenchymal transition signature in lung adenocarcinoma, which in turn correlated with upregulation of multiple suppressive immune checkpoint receptors or their ligands, including B7-H3, CTLA-4, PD-1, PD-L1, PD-L2, B and T-lymphocyte attenuator, and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3). These cumulative studies[61,91–95] strongly suggest that signalling cues imposed by attacking T-cell infiltrates during therapy-induced and natural immunosurveillance might drive cancer plasticity and therefore enable immune tolerance.

Enteric microbiome

The intestinal microbiota is increasingly recognised to interact with therapeutic outcomes for a host of disease conditions, including obesity, multiple sclerosis, arthritis, and psoriasis, albeit in complex and poorly understood ways. Several human and animal studies[96–100] have shown that gut-residing commensal bacteria might likewise dictate the efficacy of immune checkpoint blockers in cancer immunotherapy.

The intriguing associations between microbiome diversity and non-response to immune checkpoint inhibitors have been recently described.[96,97] In one study,[96] 80 patients with metastatic renal cell carcinoma prospectively treated with anti-PD-1 or anti-PD-L1 (n=67), anti-PD-1 plus anti-CTLA-4 (n=10), or anti-PD-L1 plus bevacizumab (n=3), were retrospectively analysed for treatment outcomes following antibiotic usage. Of these patients, 16 were prescribed antibiotics (mostly β-lactams and fluoroquinolones) up to 1 month before the first injection of immune checkpoint inhibitors. In the multivariable analysis adjusting for risk factors,[96] including risk group and performance status, the proportion of patients achieving disease control was significantly lower in patients treated with antibiotics than it was in those patients who did not receive antibiotics (51 of 63 patients *vs* five of 16 patients, p<0·001). These findings are reminiscent of an earlier study,[98] which observed that mice treated with broad spectrum antibiotics (ampicillin plus colistin plus streptomycin) or bred in germ-free conditions did not respond to CTLA-4 blockade. Although these studies suggest that some antibiotics could impair immunotherapy efficacy, other antibiotics might potentially augment therapeutic response. For instance, vancomycin appears to enhance the efficacy of CTLA-4 blockade in mice by decreasing the abundance of Gram-positive bacteria while preserving the Gram-negative orders Bacteroidales and Burkholderiales.[98]

In another study,[97] a bacterial signature associated with an increased abundance of Bacteroidales bacteria, but

diminished titres of *Faecalibacterium prausnitzii* and Clostridiales (specifically the Ruminococcaceae family) and low microbial diversity was predictive of primary resistance to PD-1 blockade. However, the mechanisms by which enteric bacteria modify the adjuvanticity of immune response during immunotherapy remain largely unresolved, although it has been suggested that potential cross-reactivity between microbial and tumour antigens might enhance dendritic-cell priming.[98,99,101] Based on our limited understanding, it seems unlikely that any rational approaches to salvage therapy are currently possible. However, oral administration of a commercially available cocktail of *Bifidobacterium* spp (including *Bifidobacterium breve* and *Bifidobacterium longum*) has been shown to synergise with anti-PD-L1 therapy in a mouse model of melanoma.[99]

Cooption of alternative immune checkpoints

During checkpoint blockade with anti-CTLA-4 and anti-PD-1/PD-L1 inhibitors, multiple inhibitory checkpoints might become coordinately upregulated because of interferon signalling[55,102] and activation of various pathways[103] (eg, phosphoinositide 3-kinase-AKT) in tumour-infiltrating lymphocytes, eventually leading to therapeutic failure. For instance, TIM-3 upregulation has been detected in growing lesions from patients with lung adenocarcinoma who initially had a partial response to PD-1 blockade.[104] TIM-3 was predominantly found on a PD-1 antibody-bound subset of T cells and its coexpression correlated significantly with the duration of PD-1 blockade, signifying adaptive resistance.[104] Compensatory upregulation of alternative checkpoints (eg, PD-1, lymphocyte-activation gene 3, 2B4, T-cell immunoreceptor with immunoglobulin and ITIM domains, TIM-3, CD160, and V-domain immunoglobulin suppressor of T-cell activation) has also been documented in response to single-agent immune checkpoint inhibitors in preclinical models, including head and neck cancer,[103] metastatic ovarian cancer,[105] metastatic melanoma,[55] lung adenocarcinoma,[104] and prostate cancer.[106] Multiple trials testing the utility of combination immunotherapies are underway, and some early encouraging results have been reported (table). For future research, it is essential that the expanding catalogue of immune inhibitory checkpoints that could be implicated in adaptive resistance to PD-1/PD-L1 and CTLA-4 blockade continue to be incorporated into combinatorial or sequential immunotherapies in an informed manner.

Conclusion

The advent of checkpoint blockade immunotherapy has revolutionised treatment frameworks for many malignancies. However, the major limitation of single-agent immune checkpoint blockade is the ubiquity of primary resistance and the emergence of acquired resistance in a subset of patients who show a durable response. We therefore envisage that the next decade of research will focus on making conceptual progress to rationalise and broaden the utility of immune checkpoint targeting strategies.

In this Review, we elaborated a conceptual framework to distil the complexity of cancer cell autonomous and extrinsic mechanisms that have been discovered to aid the formation of immunoresistant niches. The mechanisms of acquired resistance appear to closely parallel those operating in primary resistant tumours. Although, broadly speaking, it is clear that cancers have a range of adaptive programmes to circumvent effective immunotherapy, our understanding of the detailed pathogenetic mechanisms that orchestrate these programmes remains somewhat superficial and speculative. For example, points of conjecture exist as to how the enteric microbiome dictates immunotherapy outcomes, and whether, or to what extent, antecedent and concomitant therapies (eg, antibiotics, mitogen-activated protein kinase inhibitors, and immunogenic chemotherapy) affect the responsiveness of tumours to immune checkpoint blockers. Furthermore, although studies have traditionally focused on responders and non-responders, analysis of tumour samples from patients with long-term disease stabilisation might afford unique insights into the evolution of immunotherapeutic resistance. Likewise, detailed characterisation of multiple lesions in the setting of a mixed response might prove useful for gaining biological insights into how heterogeneous mechanisms of response and resistance can coexist within an individual patient. Beyond immunotherapeutic resistance, other challenges associated with cancer immunotherapy have emerged and should be addressed, including the optimal management of treatment-related autoimmunity[107] and the immunological basis of tumour hyperprogression in some patients treated with anti-PD-1/PD-L1.[108]

A limitation of this Review is that it does not explicitly address the scope and utility of biomarkers for predicting a patient's likelihood of being refractory to or acquiring resistance to immunotherapy. Instead, the set of concepts

Search strategy and selection criteria

We identified references for this Review through searches of PubMed between Sept 16, 2016, and May 10, 2017. We searched for articles published between Jan 1, 2008, and May 1, 2017, using the search terms "resistance", "relapse", "recurrence", "cancer immunotherapy", "checkpoint blockade", "anti-PD-1", "anti-PD-L1", and "anti-CTLA-4". We focused on the mechanistic aspects of resistance to immune checkpoint targeting and potential strategies to reverse resistance, and reviewed both preclinical and clinical data. References were also identified from the authors' own files and through reference lists of included articles. Only papers published in English were included.

enumerated might better serve as a general framework for the development of mechanism-based biomarkers against the backdrop of this rapidly changing research avenue. Furthermore, this Review serves to enrich, but not to replace, previously proposed frameworks for conceiving tumour immunity status, such as the classification of cancers based on T-cell infiltration and PD-L1 and the cancer immunogram.[79,109–111] Ultimately, this multiplicity of viewpoints will help to propel the next decade of research, and the rapid translation of these insights to the clinic might eventually allow immunotherapy to treat a greater subset of patients.

Contributors

All authors contributed to the design, writing, and revision of the Review.

Declaration of interests

TSKM reports receiving consulting fees from AstraZeneca, Roche/Genentech, Pfizer, Eli Lilly and Company, Boehringer Ingelheim, Merck Serono, Merck Sharp & Dohme, Novartis Pharmaceuticals, SFJ Pharmaceuticals, ACEA Biosciences, Vertex Pharmaceuticals, Bristol-Myers Squibb, geneDecode, Oncogenex, Celgene, Ignyta, and Cirina. TSKM also reports research sponsorship from AstraZeneca, Boehringer Ingelheim, Pfizer, Novartis Pharmaceuticals, SFJ Pharmaceuticals, Roche, Merck Sharp & Dohme, Clovis Oncology, Bristol-Myers Squibb, Eisai, and Taiho. TSKM owns stock in Sanomics and Cirina. TSKM has received honoraria from AstraZeneca, Roche/Genentech, Pfizer, Eli Lilly and Company, Boehringer Ingelheim, Merck Sharp & Dohme, Novartis Pharmaceuticals, Bristol-Myers Squibb, and Taiho, and is employed by The Chinese University of Hong Kong. RAS has received honoraria from AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Eli Lilly and Company, Merck, Novartis, Pfizer, Roche, and Taiho, and has received research funding from AstraZeneca. All other authors declare no competing interests.

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