

Mechanisms of Resistance to Immune Checkpoint Blockade

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Abstract

Immune checkpoint inhibitors (ICIs) have changed the treatment landscape of multiple malignancies. However, only around 20% of patients respond to single-agent ICI, and a limited number experience durable responses to immunotherapy. Patients may present either *de novo* (inherited) resistance to ICIs, or adaptive (acquired) resistance, limiting the efficacy of these agents. Furthermore, mechanisms of resistance can be either intrinsic or extrinsic to cancer cells. The intrinsic causes include low immunogenicity, genetic alterations, changes in cell signaling, and upregulation of alternate immune checkpoints. Extrinsic mechanisms of evasion relate to immune suppression of the tumor microenvironment by cytokines and regulatory cells. As our understanding of the complex mechanisms that result in tumor immune resistance continues to advance, various strategies, such as adoptive cell therapy, and targeting of new checkpoints and molecules, are being assessed to overcome this resistance. Additionally, different combinations of ICIs with other molecules with non-overlapping mechanisms of action (e.g., chemotherapeutic agents, antibody drug-conjugates, targeted therapies, dual ICI-ICI combinations) are currently undergoing investigation in various malignancies. In this review, we aim to provide a comprehensive overview of the different mechanisms of resistance and the various strategies employed to overcome this resistance.

Take Home Messages

1. Resistance to immune checkpoint blockade could arise through distinct cell-intrinsic pathways (e.g., neoantigen loss, antigen presentation defects, signaling alterations etc.) and cell-extrinsic factors (e.g., immunosuppressive tumor microenvironment, cytokines, and gut microbiome variations).
2. Tumor mutational burden and PD-L1 expression remain imperfect across malignancies, highlighting the need for novel biomarkers.
3. Overcoming primary and acquired resistance requires multi-agent/multimodal approaches such as combining immune checkpoint inhibitors with targeted therapies, chemotherapy, radiation, cytokine prodrugs, or novel modalities like antibody-drug conjugates and T-cell engagers.

1. Introduction

The immune system plays a protective role in preventing carcinogenesis, but various inhibitory checkpoints can hinder the immune response and facilitate tumor progression.¹ Immune checkpoint inhibitors (ICIs) are novel agents that have dramatically changed the treatment landscape of various malignancies in recent years. They function by blocking immunosuppressive interactions between tumor cells and the host's immune system, thereby enhancing the immune-mediated killing of cancer cells. The first ICI, namely ipilimumab, garnered regulatory US Food and Drug Administration (FDA) approval in 2011 for the treatment of metastatic melanoma.² To date, several ICIs with different antigen targets have been approved by the FDA across various cancers.³⁻⁵ However, a substantial number of patients do not experience any benefit from these regimens. The objective response rate (ORR) to immune targeting agents can vary widely, ranging from 16% in patients with single-agent ICIs in gastro-esophageal junction carcinoma to 58% combination immunotherapy in advanced melanoma.^{6,7} This variability in response highlights the urgent need to understand resistance mechanisms and develop biomarkers that could predict response to ICIs. In this context, resistance to immunotherapy can be classified as either primary (*de novo*) or acquired (adaptive) based on the

timing of its onset. Patients with certain neoplasms, including pancreatic cancer and glioblastoma, present primary resistance and do not respond to immunotherapy, while patients with acquired resistance initially respond before developing resistance mechanisms that limit the antitumor activity of these therapies.⁸ These instances have been encountered in melanoma, as well as bladder and kidney cancers.⁸ Furthermore, mechanisms of resistance can be either intrinsic or extrinsic to tumor cells (**Figure 1**). In this review, we provide a comprehensive overview of the mechanisms of resistance to immunotherapy and the ongoing efforts to overcome them, thereby increasing the number of patients who could potentially benefit from these therapies.

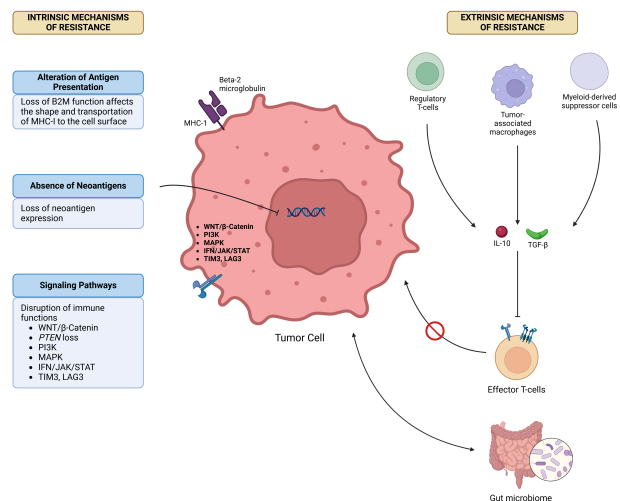


Figure 1. Intrinsic and extrinsic mechanisms of resistance to immune checkpoint blockade

Abbreviations: IFN/JAK/STAT, interferon/Janus kinase/signal transducer and activator of transcription; IL-10, interleukin 10; LAG3, lymphocyte activation gene 3; MAPK, mitogen-activated protein kinase; MHC-1, major histocompatibility complex class I; PI3K, phosphoinositide 3-kinase; TGF-β, transforming growth factor beta; TIM3, T-cell immunoglobulin and mucin-domain containing-3.

2. CTLA-4 AND PD-1/PD-L1 Immune Checkpoints

The T-cell receptor (TCR) and the major histocompatibility complex (MHC) interaction is crucial for T-cell activation. Additionally, the interaction between CD28 on T-cells and B7-1 or B7-2 on antigen-presenting cells (APCs) is necessary for T-cell activation, proliferation, and differentiation

by inducing the secretion of growth factors such as interleukin 2 (IL-2).⁹ Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which is expressed on effector T-cells (Teff) and regulatory T-cells (Tregs), competes with CD28 for B7 binding and suppresses T-cell activation, proliferation, and IL-2 production.⁹ The balance between CD28/B7 and CTLA-4/B7 interactions determines whether T-cells will undergo activation or anergy.⁹ CTLA-4 is primarily located intracellularly in a resting state, and its expression is upregulated following T-cell activation to create a negative feedback loop.⁹ On the other hand, Tregs exert their immunosuppressive effects by expressing CTLA-4 on their surface. CTLA-4 inhibitors block the CTLA-4/B7 interaction, thereby favoring the T-cell-mediated killing of cancer cells. Tremelimumab became the latest drug in this class to be approved for the treatment of advanced unresectable hepatocellular carcinoma and non-small cell lung cancer (NSCLC) in combination with durvalumab (programmed cell death-ligand 1 [PD-L1] inhibitor).^{10,11}

The interaction between programmed-cell-death-protein-1 (PD-1) expressed on T-cells and its ligand (PD-L1) expressed on APCs and tumor cells regulates the immune response.¹² When the PD-1 and PD-L1 interaction occurs, it disrupts the intracellular signaling of Teffs and Tregs, leading to a state of T-cell malfunction referred to as T-cell exhaustion, and a reduction in the secretion of key Teffs activating cytokines, including interferon- γ (IFN- γ), tumor necrosis factor- α , and IL-2.¹² Blocking the PD-1 axis restores Teffs in the tumor microenvironment (TME) and reverses T-cell exhaustion.¹³ The clinical efficacy of PD-1/PD-L1 inhibition has led to the approval of several PD-1 inhibitors (e.g., nivolumab, pembrolizumab, cemiplimab, retifanlimab, dostarlimab, toripalimab, tislelizumab) and PD-L1 inhibitors (e.g., atezolizumab, durvalumab, avelumab and cosibelimab) for a wide range of indications.³⁻⁵

3. Intrinsic Mechanisms of Resistance

3.1 Absence of Neoantigens

Intrinsic mechanisms of resistance arise from tumor cells, promoting immunity evasion. One of the key factors is the absence of immunogenic antigens on the surface of tumor cells, which makes them less susceptible to immune attack.¹⁴ Neoantigens,

peptides generated from somatic mutations unique to the tumor, can serve as immunogenic targets. A high tumor mutational burden (TMB) results in more neoantigens expressed on the tumor cell surface, leading to more cytotoxic interactions with immune cells. The presence of a high TMB was predictive of response to ICIs in certain types of cancer, including NSCLC and metastatic castration-resistant prostate cancer.¹⁵ Furthermore, in 2020, pembrolizumab acquired tumor-agnostic approval in adult and pediatric patients with high TMB (≥ 10 mutations/megabase). However, the predictive ability of TMB to ICIs could not be validated across all types of cancer. For instance, in colorectal cancer, it was demonstrated that proficient mismatch repair tumors with high TMB remained largely resistant to ICIs.¹⁶ In contrast, in renal cell carcinoma (RCC) and Merkel cell carcinoma, a high response rate to ICIs was reported despite lower TMB levels.¹⁵ This discrepancy could be attributed to the higher production of antigens resulting from insertional mutations, specifically frameshift insertional mutations.¹⁷ These mutations create novel genomic reading frames that generate unique neoantigens that are recognized as foreign by the immune system, leading to the activation of T-cells and stimulation of an immune response. High levels of insertional mutations have been demonstrated to have a predictive effect for response to ICI therapy in patients with NSCLC and RCC.^{18,19} In contrast, reduced neoantigen expression on cancer cells has been identified as a factor leading to ICI resistance.²⁰ Prostate and pancreatic cancers, which are characterized by low TMB and neoantigen expression, have been historically labeled as "cold tumors" and display limited benefit to ICI therapy. In a recent study of the neoantigen landscape of a patient with NSCLC with disease progression on PD-L1 and CTLA-4 inhibitors, analyses showed that 7 out of 18 neoantigens were lost upon disease progression, suggesting that the constant pressure from the immune system may result in the selection of a subclone with reduced neoantigen expression, rendering it resistant to immune attack.²¹ The survival of this weakly immunogenic subclone leads to tumor heterogeneity and decreased response to immunotherapy. It is noteworthy that defective homologous recombination DNA repair genes, such as *BRCA1* and *BRCA2*, increase TMB and are positively correlated with higher neoantigen expression.²² Higher *BRCA2* mutations were found in

patients with melanoma with disease response to ICIs.²³ Similar findings were also reported with other DNA damage response and repair (DDR) genes, including *ATM*, *POLE*, *ERCC2*, *FANCA*, and *MSH6*, which increase the mutational load and result in an enhanced response to anti-PD-1/PD-L1 therapies in patients with advanced urothelial carcinoma.²⁴

3.2 Antigen Presentation Alterations

Cancer cells can evade immune surveillance through mutations in the *beta-2-microglobulin* (*B2M*) gene, which encodes an extracellular component of the major histocompatibility complex I (MHC-I) expressed on all nucleated cells in the human body.²⁵ MHC-I molecules are crucial for immune recognition and for presenting cancer cell antigens to CD8+ T-cells. The protein B2M also plays a role in MHC-I folding and transportation to the cell membrane.²⁵ Loss of B2M function affects the shape and transportation of MHC-I to the cell surface. Mutations in *B2M* were detected in patients with metastatic melanoma who acquired resistance to anti-PD-1 therapy.²⁶ Similarly, loss of heterozygosity in the human leukocyte antigen (HLA) gene can down-regulate MHC expression, as reported in 40% of patients with NSCLC.²⁵ Modifications in MHC-I that affect binding to T-cell receptors have also been found in colorectal cancer.²⁷ The expression of non-classical MHC-I, such as HLA-G, can evade immunity by impairing T-cell and natural killer (NK) cell cytotoxicity.

3.3 Signaling Pathways

The role of signaling pathways in immune regulation is crucial. The WNT/ β -Catenin signaling pathway plays a part in gene transcription, organogenesis, and tumorigenesis. When β -catenin is stabilized, the WNT pathway is persistently activated, resulting in tumor resistance to ICIs and increased metastatic potential.²⁸ Additionally, up-regulation of β -catenin decreases the number of T-cells infiltrating the tumor, which is brought about by downregulation of CCL4. This vital chemokine plays a key role in recruiting dendritic cells (DCs). Without the activation of DCs, T-cells are not primed, and the immune response is suppressed. In a mouse model, tumors expressing β -catenin had a poorer response to ICI compared to β -catenin-negative tumors.²⁸ Similarly, non-T-cell-inflamed

melanomas resistant to anti-PD-L1/CTLA-4 therapies had higher expression of β -catenin signaling genes.

The *PTEN* gene plays a critical role in cancer development and response to immunotherapy. Loss of *PTEN* function activates the phosphatidylinositol 3-kinase (PI3K) signaling pathway, leading to cellular transformation, tumorigenesis, and cancer progression.²⁹ Studies have shown that *PTEN* loss reduced the expression of key components of the anti-tumor immune response, such as IFN- γ and granzyme B.²⁹ *PTEN* loss in tumor cells led to increased expression of immunosuppressive cytokines, which reduced T-cell infiltration in tumors. Additionally, it inhibited autophagy, thereby decreasing T-cell-mediated tumor cell death.²⁹ Results from a patient cohort with glioblastomas showed that those with disease progression on anti-PD-1 therapy had a higher frequency of *PTEN* mutations.³⁰

The mitogen-activated protein kinase (MAPK) pathway has a vital role in cell functions such as proliferation, apoptosis, survival, and motility, which are essential for tumor cell survival.³¹ MAPK activation, however, has also been linked to the upregulation of pro-tumor factors such as vascular endothelial growth factor (VEGF) and cytokines that impair T-cell recruitment and cytotoxicity, thereby facilitating immune evasion. On the other hand, using MAPK inhibitors has been shown to improve the function of tumor-infiltrating lymphocytes, boost MHC-I expression, and increase IFN- γ secretion.³¹

Interferons (IFNs) are a class of cytokines released to activate the immune system in response to viral infections. IFNs have a range of functions that include the recruitment of different immune cells, the inhibition of cell proliferation, and the induction of apoptosis in malignant cells.³² T-cells produce IFN- γ , which plays a crucial role in activating the immune response. IFN- γ increases the expression of MHC-I via the JAK/STAT signaling pathway, thereby increasing antigen presentation.³² However, prolonged exposure to IFN- γ can produce a negative feedback loop that suppresses the immune response by upregulating the expression of PD-L1.³² Additionally, the extended exposure of tumor cells to IFN- γ can lead to gene alterations in *JAK-1*, *JAK-2*, and *STAT*, interfering with IFN- γ signaling and decreasing antigen presentation, thereby lead-

ing to resistance to ICIs. Mutations in the IFN/JAK/STAT signaling pathway can result in resistance to anti-PD-1/PD-L1 therapy.³³

The resistance to ICIs has also been linked to the upregulation of alternate inhibitory immune checkpoints. Elevated expression of T-cell immunoglobulin, mucin-domain-3 protein (TIM-3), and lymphocyte activation gene 3 (LAG3) has been associated with resistance to immunotherapy.³⁴ For example, TIM-3, also known as hepatitis A virus cell receptor-2 (HAVCR2), an inhibitory T-cell receptor, was identified in two patients with lung cancer resistant to anti-PD-1 therapy.³⁴ New inhibitory immune checkpoint pathways such as B and T lymphocyte attenuator (BTLA), T-cell immunoreceptor tyrosine-based inhibition motif domain (TIGIT), and V-domain immunoglobulin-containing suppressor of T-cell activation (VISTA) have also been detected in the tumor microenvironment.³⁴ An upregulation of VISTA, a negative feedback mechanism, was observed on CD-4, CD-8, and M2 subtype macrophages in patients with prostate cancer treated with anti-CTLA-4 therapy.³⁵ These alternate immune checkpoints may contribute to ICI resistance by suppressing the activity of T-cells and NK cells, leading to T-cell exhaustion and reduced immunosurveillance. In patients with NSCLC, the increased expression of PD-1, TIM-3, CTLA-4, LAG-3, and BTLA on intra-tumoral CD8+ T-cells was correlated with T-cell exhaustion and disease progression.³⁶

The upregulation of PD-L1 on tumor cells or adjacent cells can suppress the activity of T-cells, resulting in tumor growth and immune resistance. It is noteworthy that high PD-L1 expression has been linked with a favorable response to pembrolizumab therapy in patients with advanced NSCLC.³⁷ However, an analysis of primary studies leading to 45 FDA drug approvals in various cancer types revealed that PD-L1 expression was predictive of response in 28.9% of cases and was not tested or predictive in 53.3% and 17.8% of cases, respectively.³⁸

Another frequently mutated pathway that contributes to cancer progression is the RAS/RAF signaling axis. RAF signaling in tumors has been shown to promote immune tolerance and evasion by reducing MHC-I expression and increasing PD-L1 expression.^{39,40} Based on preclinical studies suggesting an immune activation induced by BRAF

inhibitors, the combination of atezolizumab (PD-L1 inhibitor), vemurafenib (BRAF inhibitor) and cobimetinib (MEK inhibitor) has been approved in patients with melanoma harboring *BRAF* alterations after showing antitumor activity in a phase 3 trial.^{41,42} On the other hand, RAS is a molecular target that was shown to play a role in immune evasion by increasing PD-L1 expression, enhancing T-cell conversion into Tregs and preventing the intratumoral infiltration of T-cells.^{43–45} **Table 1** summarizes the mechanisms of intrinsic resistance to ICIs.

4. Extrinsic Mechanisms of Resistance

4.1 Immunosuppressive Cells in the Tumor Microenvironment

The TME encompasses the various components that surround and support the tumor cells growth, including molecules, cells, stroma, and blood vessels. It also substantially influences the growth of cancer cells and their response to ICIs due to the presence of immune cells and cytokines involved in immune regulation.^{46,47} Tregs are crucial in maintaining self-tolerance, preventing excessive immunity, and avoiding autoimmunity. They can inhibit the function of Teffs either through direct physical contact or by secreting inhibitory chemokines, such as IL-10, IL-35, and transforming growth factor-beta (TGF- β).^{46,47} A high ratio of Tregs compared to cytotoxic Teffs in the TME suggests a suppressed immune state. In the CheckMate-025 trial in kidney cancer, where patients with advanced or metastatic clear cell renal cell carcinoma were treated with either nivolumab or everolimus (a mammalian target of rapamycin inhibitor), the presence of a high percentage of Tregs expressing PD-1 was associated with poorer outcomes of response in the nivolumab arm. Furthermore, the investigators developed an integrated biomarker, the PD-1 Treg/CD8 ratio, which represents the ratio between the percentage of PD-1⁺ Tregs (a marker of resistance) and the percentage of CD8⁺ PD-1⁺ TIM-3⁻ LAG3⁻ T cells (a marker of response). Patients in the nivolumab arm with a high ratio experienced significantly shorter progression-free survival, overall survival, and ORR.⁴⁸ Other components of the TME that could contribute to immune evasion include cancer-associated fibroblasts, which secrete immunosuppressive cytokines such as TGF- β , and endothelial

Table 1. Intrinsic mechanisms of resistance to immune checkpoint blockade

Mechanism	Description	Mediators	Effects
Absence of neoantigens			
Absence of neoantigens	Tumor cells lack immunogenic antigens, reducing immune system detection	Insertional mutations, <i>BRCA1/BRCA2</i>	Reduced cytotoxic response, immune evasion
Low TMB	Leads to reduced neoantigen expression	TMB < 10 mutations/megabase	Reduced T-cell activation
Loss of neoantigen expression	Loss of specific neoantigens during disease progression	Immune system pressure	Tumor heterogeneity, resistance to ICIs
Antigen presentation alterations			
Mutations in the <i>B2M</i> gene	Disrupts MHC-I function, reducing antigen presentation to CD8+ T-cells	B2M, MHC-I	Impaired immune recognition, resistance to ICIs
Loss of HLA heterozygosity	Reduces MHC-I expression, impacting immune detection	HLA gene	Decreased T-cell activation
Non-classical MHC-I expression	Impairs T-cell and NK cell cytotoxicity	HLA-G, HLA-E	Immune evasion, T-cell and NK cell suppression
Signaling pathways			
WNT/ β -Catenin pathway	Decreases T-cell infiltration and dendritic cell activation	β -Catenin, CCL4	Reduced immune response, decreased response to ICI
PTEN/PI3K pathway	Reduces anti-tumor immune response and increases immunosuppressive cytokines	Loss of <i>PTEN</i>	Reduced T-cell infiltration, inhibited autophagy
MAPK pathway	Impairs T-cell recruitment and increases immunosuppressive factors	RAS, RAF, MEK, ERK; AP-1-regulated cytokines	Immune evasion, impaired T-cell recruitment and cytotoxicity
IFN/JAK/STAT pathway	Prolonged exposure leads to immune suppression	IFN- γ , JAK-1/2, STAT	Decreased antigen presentation, ICI resistance
Alternative immune checkpoints	Promote T-cell exhaustion and immune resistance	TIM-3, LAG-3, BTLA, VISTA	Suppressed T-cell activity, reduced immunosurveillance
PD-L1 expression and resistance			
PD-L1 overexpression	Suppresses T-cell activity, facilitating tumor growth	PD-L1	Tumor growth, immune resistance

M2 TAMs involvement	Increases PD-L1 expression and anti-inflammatory cytokines secretion	M2 TAMs	Reduced efficacy of PD-1/PD-L1 inhibitors
RAS/RAF signaling	Promotes immune evasion by altering immune cell activity	RAF, PD-L1, RAS	Reduced MHC-I expression, increased Treg conversion

Abbreviations: B2M, beta-2-microglobulin; BTLA, B and T lymphocyte attenuator; CCL4, C-C motif chemokine ligand 4; HLA, human leukocyte antigen; ICIs, immune checkpoint inhibitors; IFN, interferon; LAG-3, lymphocyte-activation gene 3; MHC-I, major histocompatibility complex class I; NK, natural killer; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TAMs, tumor-associated macrophages; TMB, tumor mutational burden; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; Treg, regulatory T-cells; VEGF, vascular endothelial growth factor; VISTA, V-domain Ig suppressor of T-cell activation

cells of the tumor vasculature, which promote CD8⁺ T-cell apoptosis and the differentiation of immune cells (e.g., macrophages, Tregs) into an anti-inflammatory phenotype.^{46,47}

The expression of Neuropilin-1 (NRP-1) on Tregs has been shown to play a role in their survival and suppression of the immune response to cancer cells. The presence of NRP-1-positive Tregs has been associated with a poorer prognosis in patients with cancer.⁴⁹ Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of cells that play a role in immune regulation in the TME and can contribute to tumor progression by suppressing the immune response.⁵⁰ MDSCs exert their immunosuppressive effects through the secretion of cytokines such as TGF- β and IL-10, enzymes like IDO and aminopeptidase, or reactive oxygen and nitrogen species.⁵⁰ An increased proportion of MDSCs in the TME has been associated with a poorer response to ICIs in patients with metastatic melanoma.⁵⁰ Studies in mice have shown that the PD-L1 blockade can activate CD8⁺ T-cells and initiate a PD-L1-NLRP-3 cascade that recruits granulocytic MDSCs. Inhibition of NLRP-3, either pharmacologically or genetically, has been shown to decrease the infiltration of granulocytic MDSCs into the tumor and increase the effectiveness of PD-1 blocking therapy.⁵¹ Similarly, targeting death receptor 5 (DR5), a receptor of TNF-related apoptosis-inducing ligand (TRAIL) expressed on MDSCs, with an agonistic antibody in combination with anti-PD-L1 has been shown to result in higher CD8⁺ T-cell numbers and increased anti-tumor immunity in the TME in mouse models of gastric and colon cancer. This combination also induced memory immunity and resulted in complete tumor regression.⁵¹ Additionally, targeting TYRO3, AXL, and MERTK on MDSCs has been

demonstrated to improve the response to anti-PD-1 therapy in melanoma.⁵²

Tumor-associated macrophages (TAMs), specifically the M2 subtype, are abundant in the TME. They support a conducive environment for tumor proliferation by producing immune-suppressive cytokines, such as IL-10 and TGF- β , which inhibit the activity of Tefs, attract Tregs, and impair the maturation of DCs, resulting in reduced antigen presentation.⁵² In kidney cancer, M2-like TAMs interact with terminally exhausted CD8⁺ T-cells to cause an immune dysfunction associated with a poor prognosis.⁵³ Terminally exhausted CD8⁺ T-cells interacted with M2-like TAMs through cytokine and chemokine signaling and cell adhesion. Several predicted interactions involved T-cell inhibitory signaling or the suppression and M2-like polarization of macrophages. M2-like TAMs expressed ligands for numerous T-cell immune checkpoints (e.g., *PDCD1LG2*/PD-L1, which binds to *PDCD1*/PD-1). Additionally, terminally exhausted T-cells also expressed genes for ligands that support M2-like polarization (e.g., *MIF*, which binds to CD74). A recent study reported a correlation between high histamine levels and high expression of the histamine receptor H1 (HRH1), and resistance to immunotherapy through polarization of macrophages towards the M2 phenotype. Patients with low plasma histamine levels demonstrated a better ORR to anti-PD-1 therapy compared to those with high plasma histamine.⁵⁴ Colony-stimulating factor-1 (CSF-1) is a crucial factor in TAM recruitment. The combination of anti-PD-1 and anti-CSF-1 therapy in mouse models resulted in regression of BRAF-driven melanoma.⁵⁵ On the other hand, macrophages can also contribute to pro-immunological defense, as certain chemother-

apy agents, such as doxorubicin, release tumor antigens that activate macrophages to perpetuate the immune response.⁵⁶

4.2 Cytokines in the Tumor Microenvironment

Several cytokines in the TME have been demonstrated to contribute to immune evasion. In a mouse model, the combination of TGF- β antagonist and PD-L1 antibodies resulted in increased infiltration of effector T-cells into the tumor, leading to tumor shrinkage.⁵⁷ Elevated levels of pro-angiogenic factors such as VEGF and angiopoietin-2 (ANG2) are commonly found in solid cancers and contribute to tumor immune evasion by promoting vascular abnormalities.⁵⁷ Anti-vascular agents that target these factors normalize the tumor vasculature, allowing effector T-cells to penetrate the tumor and improve the immunogenicity of the TME.⁵⁷ A previous study showed that responders to ICIs have lower VEGF levels than non-responders.⁵⁸ Additionally, ICIs can repair the tumor vasculature, creating a positive feedback loop that further enhances T-eff infiltration and normalization of tumor vessels, resulting in a more robust anti-tumor immune response.⁵⁸

4.3 Gut Microbiome and Treatment Modifiers

There is growing interest in the relationship between gut microbiome and immune regulation. Though not yet fully understood, the gut microbiome is believed to influence host immunity and cancer development by positively and negatively interacting with various established hallmarks of cancer.⁵⁹ In a mouse melanoma model, oral *Bifidobacterium* administration led to a similar anti-tumor effect as treatment with PD-L1 blockade.⁵⁹ In human studies, an enhanced response to anti-PD-L1 therapy was positively correlated with the abundance of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* in the gut microbiome.⁵⁹ In addition, an increased abundance of *Ruminococcaceae* in the gut microbiome was associated with improved response to PD-1 blockade immunotherapy.⁵⁹ Additionally, in more recent work, investigators have retrieved the fecal metagenomes from patients with distinct cancer types treated with ICIs in an attempt to increase the understanding of the relationship between the

gut microbiome (including non-bacterial microbes) and response to ICIs.⁶⁰ They were able to identify *trans*-kingdom microbial biomarkers that could predict response to ICIs. For instance, bacterial species such as *Faecalibacterium prausnitzii*, and *Coprococcus comes*, as well as eukaryotes such as *Nemania serpens*, and *Hyphopichia pseudoburtonii* were enriched in patients with distinct cancer types who responded to ICIs, whereas the bacterium *Hungatella hathewayi* were depleted in these patients.⁶⁰

In the first-line metastatic RCC setting, one of the live bifidogenic bacterial products tested, CBM588, showed butyrogenic properties that foster the growth of *Bifidobacterium* spp, which could be associated with an improvement in the efficacy of ICIs.⁶¹ A recent study reported that the addition of CBM588 to the combination of nivolumab plus ipilimumab significantly improved progression-free survival (PFS).⁶¹ Furthermore, the addition of the same bacterial product to the combination of nivolumab plus cabozantinib (tyrosine kinase inhibitor) in a similar setting significantly improved ORR without affecting the toxicity profile.⁶² Additionally, previous reports have suggested that the gut microbiome impacts the efficacy of PD-1-based immunotherapy in epithelial tumors.⁶³ This suggests the need to validate these findings in larger studies.

An analysis of 283 patients with various types of cancer revealed that the use of antibiotics 30 days prior to immunotherapy was associated with decreased survival, possibly due to disruption of the gut microbiome components and a reduction in immunostimulatory cytokines such as IFN- γ .^{64,65} A recent work in patients with metastatic urothelial carcinoma showed that those treated with specific antibiotics within 30 and 60 days before or after ICI initiation had poorer survival outcomes.⁶⁶ These data also support a potential interaction between the gut microbiome and the antitumor activity of ICIs.

4.4 Host-Related Factors

In older patients with cancer treated with ICIs, immunosenescence is marked by a reduction in peripheral naïve T-cells, a relative increase in memory T-cells, diminished T-cell receptor repertoire, and alterations in regulatory T-cell populations. Memory T-cells derived from aged naïve T-cells

Table 2. Extrinsic mechanisms of resistance to immune checkpoint blockade

Mechanism	Description	Mediators	Effects
Tumor microenvironment (TME)	Includes cells, stroma, and blood vessels supporting tumor growth	Immune cells, cytokines (e.g., IL-10, IL-35, TGF- β)	Suppressed immune response, increased tumor growth
Regulatory T-cells (Tregs)	Maintain self-tolerance and suppress Teff function	PD-1 ⁺ Tregs, IL-10, TGF- β	Suppressed immune state, poor response to ICIs
Cancer-associated fibroblasts	Secrete immunosuppressive cytokines	TGF- β	Reduces CD8 ⁺ T-cell activity
NRP-1 expression on Tregs	Enhances Treg survival and immune suppression	NRP-1	Suppressed immune response
Myeloid-derived suppressor cells (MDSCs)	Group of cells that play a role in immune regulation in the TME	TGF- β , IL-10, IDO, reactive oxygen species	Poor response to ICIs, immune suppression
M2 subtype of tumor-associated macrophages (TAMs)	Production of immunosuppressive cytokines	IL-10, TGF- β , CSF-1	Inhibits Teffs, recruits Tregs, reduces antigen presentation
Gut microbiome	Microbes influencing gut health	Bacterial products (e.g., <i>Bifidobacterium</i> , <i>Collinsella</i> , <i>Enterococcus</i>), non-bacterial microbes	Influences host immunity and response to ICIs
Immunosenescence	Age-related decline in immune function	Reduction in naïve T-cells, diminished T-cell receptor repertoire, alterations in Tregs	Reduced efficacy of ICIs

Abbreviations: ICIs, immune checkpoint inhibitors; NRP-1, neuropilin-1; PD-1, programmed death-1; Teffs, effector T-cells; Tregs, regulatory T-cells; TGF- β , transforming growth factor beta.

show reduced proliferation and lower effector cytokine production, leading to a weaker immune response compared to memory T-cells generated from younger naïve T-cells.⁶⁷ Although older patients have been underrepresented in clinical trials assessing the efficacy of ICIs, multiple meta-analyses have pooled the results of different phase 3 clinical trials to assess the efficacy of ICIs in patients with different age subgroups.^{67–70} Their findings suggest that ICIs seem to be similarly effective in both younger and older patients; however, the benefit may be reduced in those over 75 and with anti-CTLA-4 ICIs, possibly due to age-related changes linked to immunosenescence. For younger patients, specific considerations around toxicities include issues related to infertility and contraception.^{67–70} Extrinsic mechanisms to immune checkpoint blockade have been summarized in **Table 2**.

5. Strategies of Improving Efficacy and/or Overcoming Resistance

Efforts are ongoing to address resistance to immunotherapy and to induce immunogenic response in tumors with low immunogenicity (**Table 3**). One strategy to overcome resistance to immunotherapy is to combine ICIs with other treatments, such as conventional chemotherapy, radiation therapy, or another ICI. Approval has been granted for the combination of anti-CTLA-4 and anti-PD-1 treatment in various cancers, including melanoma, RCC, and NSCLC.^{71–73} A minimum 5-year follow-up analysis of this combination has demonstrated better response rates and improved survival outcomes in patients with advanced melanoma compared to single-agent ICI treatment.⁷ Additionally, this combination has dramatically improved survival

outcomes in the first-line metastatic RCC setting, reaching a median of around 53 months for overall survival in an 8-year update of the CheckMate-214 trial.⁷⁴ This combination has recently been moved to more upfront settings, where it demonstrated a significantly longer event-free survival when administered as a neoadjuvant treatment compared to adjuvant nivolumab in patients with resectable stage III melanoma in the phase 3 NADINA trial.⁷⁵ Other ICI-based combinations are undergoing assessment to overcome the limitations of CTLA-4 – PD-1/L1 combinations. These include combinations inhibiting PD-1 and LAG-3 expressed on cancer cells that interact to suppress T-cell function and have shown promising results in the phase 2/3 randomized RELATIVITY-047 trial versus nivolumab monotherapy in patients with untreated advanced melanoma (median PFS 10.1 months vs. 4.6 months, HR 0.75, 95% CI 0.62-0.92).⁴ This led to the FDA approval of the combination therapy in this setting. Furthermore, the combination of atezolizumab with a TIGIT inhibitor, which blocks an immune checkpoint that inhibits the CD8-mediated activation of T-cells, has not demonstrated significant clinical benefit to date. TIGIT-based combinations failed to improve outcomes in NSCLC and have also not shown meaningful efficacy in advanced hepatocellular carcinoma or gastroesophageal cancers.⁷⁶⁻⁷⁹ Different other combinations have also been tested to improve the antitumor activity of ICIs. These combinations include enfortumab vedotin, a nectin-4-targeting antibody-drug conjugate, plus pembrolizumab, which have reshaped the treatment landscape of first-line advanced or metastatic urothelial carcinoma and garnered regulatory approval in this setting.⁸⁰ Also in urothelial carcinoma, the combination of cetrelimab and erdafitinib, an FGFR3 inhibitor, showed an objective response rate of 54.5%, compared to 44.2% with single-agent erdafitinib, in biomarker selected patients with cisplatin-ineligible metastatic disease.⁸¹ In a different combination, the addition of WTX-124, which is an engineered cytokine prodrug composed of a wild-type IL-2, to pembrolizumab is undergoing investigation in patients with selected advanced or metastatic solid tumors in a first-in-human trial (NCT05479812).

Combining chemotherapy or radiation with PD-1 blockade has also been approved in multiple cancers, including NSCLC, resulting in improved re-

sponse rates and survival.^{82,83} The rationale behind combining chemotherapy or radiation treatment is to heat the TME, thereby leading to an increased expression of antigens and activation of Tregs.⁸⁴ Radiation delivered to the tumor site impacts both tumor cells and the surrounding stromal cells. This damage to cancer cells exposes tumor-specific antigens, enhancing their visibility to immune surveillance and supporting the priming and activation of cytotoxic T-cells. Additionally, radiation-induced changes to the TME may aid in the recruitment and infiltration of immune cells.⁸⁴ In this context, multiple phase 3 clinical trials have demonstrated a significant benefit with the addition of an ICI in the adjuvant setting following definitive radiotherapy.⁸⁵ Examples of these trials include the PACIFIC trial, which assessed the role of adjuvant durvalumab in patients with unresectable stage III NSCLC, and the CheckMate-577 trial, which investigated adjuvant nivolumab in patients with stage II-III esophageal and gastroesophageal junction cancer.^{86,87} These trials, in addition to others, have significantly improved the survival outcomes of patients with cancer treated with ICIs, supporting further investigation of similar combinations.

Vaccines targeting neoantigen epitopes in combination with ICIs could also represent a potential approach to overcome resistance to immunotherapy (NCT03289962). Similarly, the use of oncolytic viruses, such as talimogene laherparepvec (T-VEC), a genetically modified herpes simplex virus-1 (HSV-1), is being explored to promote T-cell infiltration and antigen presentation. Although T-VEC has been approved for the treatment of advanced melanoma, after showing an increased ORR in combination with ipilimumab compared to ipilimumab alone, results from a phase III trial showed that T-VEC in combination with pembrolizumab did not result in a significant survival benefit compared to placebo plus pembrolizumab.⁸⁸⁻⁸⁹ However, a phase Ib study showed promising results with an ORR of 47% in patients with advanced melanoma receiving the combination of coxsackievirus A21 and pembrolizumab.⁹⁰

The combination of molecularly targeted therapies with ICIs is another avenue being explored to overcome resistance. In melanoma, BRAF inhibition resulted in favorable immunostimulatory changes in the TME, including increased antigen expression, T-cell infiltration, and decreased immunosuppres-

Table 3. Strategies to overcome resistance to immune checkpoint blockade

Strategy	Description	Examples
Combination of ICIs	Targeting different immune checkpoints	Anti-CTLA-4 + Anti-PD-1 (e.g., ipilimumab + nivolumab)
ICI + chemotherapy	Heats the TME and activates Teffs	PD-1 inhibitor + chemotherapy
ICI + radiation therapy	Enhances antigen presentation and T-cell recruitment and activation	PD-1/L1 inhibitors + radiation therapy
ICIs + Targeted therapies	Increased ICI response by enhancing the TME immunostimulation and T-cell infiltration	ICIs + BRAF inhibitors / PI3K- γ inhibitors / FGFR3 inhibitors
ICIs + vaccines	Vaccines targeting tumor neoantigens leading to increased T-cell function	ICIs + talimogene laherparepvec (T-VEC), a genetically modified herpes simplex virus-1 (HSV-1), coxsackievirus A21
ICIs + cytokine prodrugs	Engineered cytokines to boost ICI efficacy	Pembrolizumab + WTX-124 (engineered IL-2)
CAR-T-cell therapy	Genetically engineered T cells targeting specific tumor antigens	Autologous T-cells expressing the 19-28z CAR
T-cell engagers (TCEs)	Dual binding of CD3 on Teffs and tumor antigen targets leading to T-cell mediated killing of cancer cells	Blinatumomab (B-cell ALL), Tebentafusp (uveal melanoma), Tarlatamab (small cell lung cancer)

Abbreviations: ALL, acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T-cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ICIs, immune checkpoint inhibitors; IL-2, interleukin-2; PD-1/L1, programmed death-1/ligand-1; Teffs, effector T-cells; TME, tumor microenvironment

sive cytokines.⁹¹ In mice models, blocking the action of PI3K- γ has been shown to transform macrophages from an immunosuppressive M2 subtype to an immune-stimulatory M1 subtype and increase response to ICIs.⁹²

Another emerging strategy to overcome resistance to immunotherapy involves Fc-engineered ICIs designed to enhance effector T-cell activity while limiting immunosuppressive signaling within TME.⁹³ Botensilimab, an Fc-enhanced anti-CTLA-4 antibody, has demonstrated encouraging clinical activity with manageable safety profile in traditionally immune-cold tumors, including relapsed/refractory microsatellite-stable colorectal cancer.⁹³

In recent years, immune targeting agents with a novel mechanism of action have emerged, including chimeric antigen receptor (CAR) T-cell therapy and T-cell engagers (TCE). CAR-T therapies involve the collection, modification to target

a specific tumor antigen, and re-introduction of autologous T-cells into a patient.⁹⁴ These agents have shown promising results in hematologic malignancies, with complete remission rates of 83% in acute lymphoblastic leukemia and an ORR of 84% in refractory large B-cell lymphomas.⁹⁴ However, its efficacy has been limited in non-hematologic cancers due to difficulties in identifying appropriate targets, off-target effects, and the immunosuppressive nature of the TME.⁹⁵ On the other hand, TCE act by binding CD3 on Teffs and a specific tumor antigen on cancer cells, leading to T-cell mediated killing of tumor cells.⁹⁶ They are already part of the therapeutic armamentarium of various hematologic malignancies (eg, blinatumomab for the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia), uveal melanoma (Tebentafusp targeting glycoprotein 100 peptide) and small cell lung cancer (tarlatamab targeting delta-like ligand 3 [DLL3]).^{84,97,98} These agents have also

shown promising activity in other cancers, including prostate cancer, but have been limited by their toxicity profile. Further efforts are needed to optimize the administration strategies and to mitigate the potential side effects encountered before these therapies can be implemented in clinical practice. Other promising areas of development include advancements in data acquisition and computational analysis (i.e., omics), which aim to enhance our understanding of the underlying biology of cancer and improve the efficacy of immune-targeting agents.⁹⁹

6. Conclusion

The introduction of ICIs has revolutionized the treatment of various types of cancer by improving survival outcomes. Despite the rapid approval of these agents, only a small fraction of patients with advanced disease achieve a durable response due to primary or acquired resistance. To expand the benefits of immunotherapy to a larger patient population, efforts are being made to gain a comprehensive understanding of the mechanisms by which cancer cells evade detection and elimination by the immune system. Additionally, research is being conducted to elucidate the complex interactions between the tumor and the immune system, including the tumor microenvironment, cytokine milieu, tumor heterogeneity, and microbiome, to identify potential predictive biomarkers that could guide treatment selection and clinical decision-making.

Conflict(s) of Interest

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List of Abbreviations

HAVCR2: Hepatitis A virus cellular receptor 2

ANG2: Angiopoietin-2

BTLA: B and T lymphocyte attenuator

CAR: Chimeric antigen receptor

CCL4: C-C motif chemokine ligand 4

CSF-1: Colony-stimulating factor-1

CTLA-4: Cytotoxic T-lymphocyte associated pro-

tein 4

DC: Dendritic cells

DDR: DNA damage response

DR5: Death receptor 5

FDA: Food and Drug Administration

HLA: Histocompatibility leukocyte antigen

HRH1: Histamine receptor H1

IDO enzyme: Indoleamine 2,3-dioxygenase enzyme

IFN: Interferon

IFN-GAMMA: Interferon-gamma

ICI: Immune checkpoint inhibitor

IL-2: Interleukin-2

ITIM: Immunoreceptor tyrosine-based inhibitory motif

MAPK: Mitogen-activated protein kinase

MDSCs: Myeloid-derived suppressor cells

MHC: Major histocompatibility complex

NK: Natural killer cells

NKG2-A: Natural killer group 2 member A

NLRP-3: Nod-like receptor pyrin domain containing 3

NRP-1: Neuropilin-1

NSCLC: Non-small cell lung cancer

ORR: Objective response rate

PD-1: Programmed cell death protein 1

PD-L1: Programmed death-ligand 1

PFS: Progression-free survival

PI3K: Phosphoinositide 3-kinase

RCC: Renal cell carcinoma

TCR: T cell receptor

T-VEC: Talimogene laherparepvec

TAM: Tumor-associated macrophages

Teff: Effector T cells

TIGIT: T cell immunoreceptor with Ig and ITIM domains

TGF- β : Transforming growth factor beta

TMB: Tumor mutational burden

TME: Tumor microenvironment

TRAIL: TNF-related apoptosis-inducing ligand

Treg: Regulatory T cells

TYRO3, AXL, and MERTK: Tyrosine kinase receptors

VEGF: Vascular endothelial growth factor

VISTA: V-domain Ig suppressor of T cell activation

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