Mechanisms of Resistance to Immune Checkpoint Blockade: Why Does Checkpoint Inhibitor Immunotherapy Not Work for All Patients?

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Overview

The emergence of immune checkpoint blockade therapies over the last decade has transformed cancer treatment in a wide range of tumor types. Unprecedented and durable clinical responses in difficult-to-treat cancer histologies have been observed. However, despite these promising long-term responses, the majority of patients fail to respond to immune checkpoint blockade, demonstrating primary resistance. Additionally, many of those who initially respond to treatment eventually experience relapse secondary to acquired resistance. Both primary and acquired resistance are a result of complex and constantly evolving interactions between cancer cells and the immune system. Many mechanisms of resistance have been characterized to date, and more continue to be uncovered. By elucidating and targeting mechanisms of resistance, treatments can be tailored to improve clinical outcomes. This review will discuss the landscape of immune checkpoint blockade response data, different resistance mechanisms, and potential therapeutic strategies to overcome resistance.

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INTRODUCTION

Immunotherapy has recently become a viable option for cancer treatment; however, the concept of harnessing the immune system to fight malignancy dates back over a century. In the 1890s, Dr. William Coley observed improved clinical outcomes in patients with cancer who experienced postsurgical infections. Based on these observations, Coley experimented by introducing bacterial toxins to patients with sarcoma. Although results were inconsistent, he was able to demonstrate tumor regression in a subset of patients.[1] However, with the advent of chemotherapy and radiotherapy, immunotherapy went largely overlooked. In the 1950s, Ehrlich formulated the concept of immunosurveillance, which proposed that emergence of malignant cells is a frequent event, but evolution to clinically relevant disease is suppressed by the immune system unless immunity is weakened.[2] Although these early hypotheses fueled the field of cancer immunotherapy, better understanding of immune activation, regulation, and interaction with tumor cells and the microenvironment was needed.

Now we know that the process of T-cell–mediated immunity is a complex sequence of events, with constant interplay between stimulatory and inhibitory signals that promote adaptive responses against foreign antigens while avoiding autoimmunity. Antigen-specific T cells initially undergo clonal selection, with subsequent priming and activation following T-cell receptor recognition of corresponding antigens on major histocompatibility complexes (MHCs) expressed by antigen-presenting cells. For full activation, a costimulatory signal is needed between antigen-presenting cells and T cells. After activation and proliferation, T cells are trafficked to specific sites by following a chemokine gradient. Upon encountering cognate antigen on MHCs, effector T cells (Teffs) release interferon gamma (IFN-γ) and other cytokines, promoting cytotoxicity and tumor cell killing. Following cancer cell eradication, memory T cells form and remain quiescent until antigen re-exposure.

Under normal physiologic conditions, immune checkpoints function as negative feedback to regulate inflammatory responses following T-cell activation. The CTLA-4 immune checkpoint receptor was first characterized by Brunet et al[3] in the 1980s. Seminal work by Krummel and Allison[4] demonstrated that CTLA-4 on T cells competitively binds to B7 ligands on antigen-presenting cells, interfering with CD28 interactions, thus preventing costimulation and the priming phase of T-cell activation (Fig. 1A). Subsequently, blockade of CTLA-4 with antibodies demonstrated tumor rejection and emerged as proof of concept for immune checkpoint inhibitors.[5] Another immune checkpoint receptor, PD-1, was cloned in 1992[6] with subsequent characterization of its ligand, PD-L1.[7-9] Interaction of PD-1 with its

PRACTICAL APPLICATIONS

- Immune checkpoint inhibitors provide durable clinical responses in multiple difficult-to-treat tumor types.
- The tumor microenvironment, tumor immunogenicity, antigen presentation, and classic oncologic pathways play roles in response and resistance to immune checkpoint blockade.
- By understanding resistance mechanisms to immune checkpoint blockade, therapies can be developed to overcome resistance and treatment failure.
- Combination treatment strategies with immune checkpoint inhibitors are being tested in clinical trials, with several already in clinical use.
- Response to immunotherapy may be better predicted by using a wide set of biomarkers.

ligands, PD-L1 and PD-L2, inhibits the effector phase of T-cell activation, thus dampening the immune response.[10] Many tumors are now known to hijack this mechanism to avoid T-cell killing, and inhibitory antibodies directed against the interaction between PD-1 and its ligands have demonstrated antitumor responses.[11]

CLINICAL RESPONSE TO IMMUNE CHECKPOINT INHIBITORS

To date, seven immune checkpoint inhibitors have received U.S. Food and Drug Administration approval: one CTLA-4 inhibitor (ipilimumab), three PD-1 inhibitors (nivolumab, pembrolizumab, and cemiplimab), and three PD-L1 inhibitors (atezolizumab, durvalumab, and avelumab). Ipilimumab was the first immune checkpoint inhibitor to gain approval in 2011 for the treatment of melanoma.[12] In 2014, nivolumab and pembrolizumab were approved in melanoma and have now gained indications for use in non–small cell lung cancer (NSCLC), renal cell carcinoma, head and neck squamous cell carcinoma, urothelial carcinoma, and microsatellite instability–high colorectal cancer, among several other tumor types.[13-36] Atezolizumab, avelumab, and durvalumab are approved in many of the same histologies as the PD-1 inhibitors.[37-42] Most recently, cemiplimab was approved for treatment of metastatic cutaneous squamous cell carcinoma.[43]

One of the hallmarks of immunotherapy is the durability of the responses that can be translated into survival benefit. Indeed, in approved indications, checkpoint inhibitor immunotherapy prolonged survival in patients with responding disease, raising the tail of patient survival curves. However, only a subset of tumor histologies and a small percentage of the patients in each histology are responsive to these inhibitors. The response rates of different tumor types to PD-1/PD-L1 checkpoint blockade tend to be proportional to their corresponding tumor mutational burden (TMB), presumably from the immunogenic neoantigens that are recognized as foreign by cytotoxic T lymphocytes (CTLs).[44-48] However, tumors with similar TMB can have very different response to checkpoint inhibitors, indicating that response to immune checkpoint blockade (ICB) is complex, heterogeneous, and inconsistent and that additional mechanisms are at play. Increased PD-L1 expression has been correlated with immune response and is currently used as a biomarker for ICB therapy in NSCLC and urothelial carcinoma.[49,50] Additionally, elevated numbers of tumor-infiltrating lymphocytes (TILs) have been noted in responsive cancers.[51,52]

MECHANISMS OF RESISTANCE

The biggest challenges for the cancer immunotherapy field are to understand the complex resistance mechanisms and to develop effective combination strategies to overcome resistance. According to the timing of occurrence, resistance can be primary, as in never-responders, or acquired, which emerges after a period of response. Resistance can also be classified as intrinsic or extrinsic to tumor cells. Intrinsic resistance is seen when cancer cells alter processes that are related to immune recognition, cell signaling, gene expression, and DNA damage response. Extrinsic resistance occurs external to tumor cells throughout the T-cell activation process.

Tumor Immunogenicity

The ability for tumors to induce adaptive immune responses relies on recognition of cancer cells as foreign. High TMB, with accompanying elevated neoantigen expression, plays an important role in antitumor immunity.[44,53] With improved sequencing techniques, nonsynonymous mutations were found to generate tumor neoantigens that drive cytotoxic responses against cancer cells.[54,55] Van Allen and colleagues[52] demonstrated that mutational load was significantly associated with response to anti–CTLA-4 treatment in patients with metastatic melanoma. Additionally, Rizvi et al[45,46] showed that response to anti–PD-1 treatment correlated with high TMB and neoantigen load in patients with NSCLC. In keeping with these studies, poorly immunogenic tumors with low TMB, such as pancreatic and prostate cancers, are inherently more resistant to treatment with checkpoint inhibition.[44]

Extrapolating from these data, mechanisms leading to loss of neoantigen expression by cancer cells may result in acquired resistance to ICB. The concept of immunoediting exemplifies the impact of neoantigen loss on tumor immunogenicity and explains how resistance might be formed against cancers with high TMB. Immunoediting suggests that constant interactions between the immune system and cancer cells result in selection of subclones within the tumor

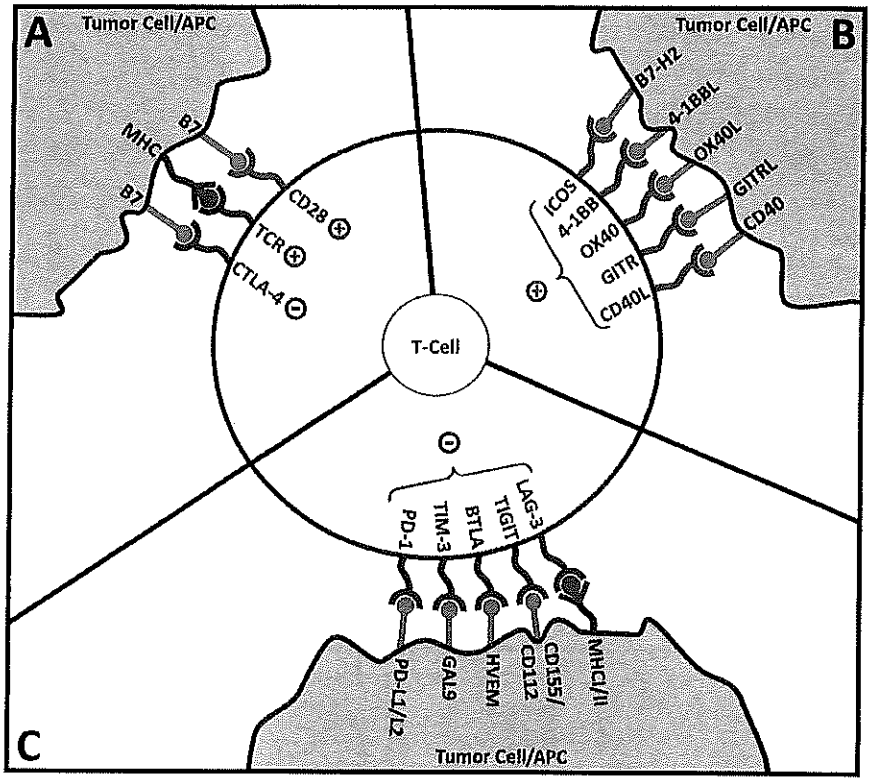

FIGURE 1. T-Cell Activation and Cosignaling

(A) T-cell receptor interacts with antigen/major histocompatibility complex on APCs. Costimulatory signal is provided by B7/CD28 interaction for T-cell activation. CTLA-4 competes with CD28 for B7 binding, providing coinhibitory signals. (B) Costimulatory signals currently being targeted to improve T-cell activation. (C) Expression of coinhibitory receptors leads to T-cell exhaustion. Coinhibitory receptors serve as therapeutic targets to enhance antitumor immune response.
Abbreviation: APC, antigen-presenting cell.

that lack expression of neoantigens, subsequently conferring poor immunogenicity and resistance to ICB.[56,57] With increased intratumor heterogeneity, there is greater likelihood that a poorly immunogenic subclone could be selected, thus decreasing sensitivity to checkpoint inhibition.[58,59] A recent study by Anagnostou et al[60] showed that relapse of NSCLC tumors after treatment with PD-1/PD-L1 and CTLA-4 inhibitors demonstrated loss of seven to 18 putative neoantigens, supporting the role of immunoediting in acquired resistance (Table 1). Another study recently showed that expression of IFN-γ paradoxically facilitates immunoediting by CTLs, with resulting gene copy number alteration contributing to immune resistance.[61]

Genetic instability due to alterations in DNA repair and replication genes can increase immunogenicity through high mutational burden with subsequent neoantigen formation. Patients with melanoma were found to have better response to anti–PD-1 treatment if tumor cells were enriched for mutations in *BRCA2*, an important homologous recombination DNA repair gene.[62] Similar findings were demonstrated in ovarian cancer, in which *BRCA1/2*-mutated tumors demonstrated high neoantigen loads.[63] Alterations in additional DNA damage response genes, including *ATM*, *POLE*, *FANCA*, *ERCC2*, and *MSH6*, have recently shown correlation with high TMB and improved clinical outcomes to ICB in urothelial cancer.[64] Furthermore, tumors with deficiencies in DNA mismatch repair genes leading to microsatellite instability demonstrated high mutational burden with enhanced response to ICB across a wide range of histologies.[65,66]

The presence of PD-L1–expressing cancer cells within tumors is known to be an important predictor of response to ICB therapy and is commonly used as a biomarker.[67] It has been shown that tumors lacking PD-L1 expression generally show inferior clinical outcomes to ICB compared with those with higher levels of ligand.[50] However, tumors with absent PD-L1 can respond to ICB, as PD-L1 expression can be induced upon activation of the IFN response pathway. Regardless of PD-L1 expression, tissues that lack TILs are unlikely to respond to ICB. Tumors with larger numbers of TILs demonstrate greater response to ICB and may serve as another predictive biomarker.[58,68-70] A study in patients with metastatic melanoma showed that pre-existing tumoral CTLs are a qualification for response to anti–PD-1 therapy.[51]

Tumor Microenvironment

The tumor microenvironment (TME) consists of factors extrinsic to cancer cells, including various immune and stromal cells, vasculature, extracellular matrix, and cytokines that influence response to therapy. Immune-suppressive cells,

TABLE 1. Mechanisms of Resistance in PD-L1–Overexpressed Tumors, Combination ICB, Tumors With Mutated Chromatin Remodeling Complexes, and High TMB Cancers

Specific Circumstances	Mechanisms of Resistance
PD-L1–overexpressed tumors	Nonreversible and severe T-cell exhaustion
	Coexpression of inhibitory receptors (LAG-3, TIM-3, TIGIT, VISTA, and BTLA)[122]
	Decreased ratio of TILs to Tregs and MDSCs
	Altered metabolism through IDO and increased adenosine production
	Mutations in *PTEN*, *EGFR*, and *MYC*[160]
PD-1 and CTLA-4 inhibitor combination therapy	Immunoediting with loss of neoantigens[60]
	Deletions or mutations in *JAK1/2*, *IFNGR1/2*, and *IRF1*[118]
	Decreased T-cell priming and DC dysfunction
	Aberrant WNT/β-catenin signaling
	High copy number loss of tumor suppressor genes[61]
Association with neoantigen overexpression by genetic alterations in mammalian SWI/SNF chromatin remodeling complexes	Loss-of-function mutations in chromatin remodeler genes (*PBRM1*, *ARID2*, and *BRD7*) sensitize tumors to ICB and increase accessibility to regulatory elements of IFN-γ–inducible genes. Loss of *ARID1A* leads to increased microsatellite instability with inability to recruit mismatch repair genes during DNA repair, increasing mutational burden and neoantigen load. Stability of chromatin remodeling complexes in tumors contributes to ICB resistance.[161-163]
High mutation overload tumors	Decreased antigen presentation secondary to MHC, β2-microglobulin, and NLRC5 alterations[164]
	JAK1/2 mutations and decreased IFN-γ signaling
	Upregulation of alternate inhibitory checkpoints

Abbreviations: TIGIT, T-cell immunoreceptor tyrosine-based inhibition motif domain; VISTA, V-domain immunoglobulin-containing suppressor of T-cell activation; BTLA, B and T lymphocyte attenuator.

along with inhibitory cytokines in the TME, can undermine the antitumor immune response.[70,71] Regulatory T cells (Tregs) are known to facilitate self-tolerance by suppressing Teff function through inhibitory cytokines and direct contact, limiting inflammation.[72,73] Infiltration of tumors by Tregs has been observed in many tumor types, suggesting an immunosuppressive environment in some cancers.[74] The ratio of Teffs to Tregs in murine models is associated with response to ICB, in that inability to increase Teffs or decrease Tregs may result in resistance to immunotherapy.[75-77]

Myeloid-derived suppressor cells (MDSCs) are another type of regulatory cell within the TME that can promote immune evasion and tumor growth.[78,79] MDSCs have been shown to play a role in facilitating tumor invasion, metastasis, and angiogenesis.[80,81] Clinical studies demonstrate that increased presence of MDSCs within the TME correlates with poor response to ICB.[82] Accordingly, by inhibiting trafficking of MDSCs to the TME, enhanced response to anti–PD-1 therapy was seen in a murine model of rhabdomyosarcoma.[83]

Tumor-associated macrophages, particularly M2 macrophages, promote tumor progression through modifications of the TME.[84] M2 macrophages are known to stimulate tumor cell motility, angiogenesis, growth, and immune evasion.[85] Consequently, depletion of tumor-associated macrophages in several different murine models correlated with reduced tumor growth.[86,87] Moreover, inhibition of myeloid growth factor signaling in macrophages circumvented therapeutic resistance to ICB in a murine model of pancreatic cancer.[88,89]

The cytokine milieu within the TME is involved in immune cell recruitment, activation, and proliferation, exerting both immune stimulatory and suppressive effects.[90] Several chemokines, including CCL5, CCL17, CCL22, CXCL8, and CXCL12, play a role in recruiting MDSCs and Tregs to the TME, thus promoting an immunosuppressive climate.[83,91] Consequently, inhibition of the chemokine receptor CCR4 diminished trafficking of Tregs and promoted antitumor effects.[92,93] Alternately, CXCL9 and CXCL10 recruit CTLs to the TME, with subsequent destruction of cancer cells.[94,95] Expression of CXCL9 and CXCL10 can be epigenetically silenced, reducing TILs and promoting resistance to ICB. Epigenetic modulator therapy in a model for ovarian cancer reversed suppression of these chemokines and enhanced response to ICB.[96]

Transforming growth factor beta (TGF-β) signaling influences multiple TME elements, including cell growth and

differentiation, wound healing, apoptosis, and immunosuppression. TGF-β limits immunosuppression through inhibition of CTLs while upregulating Tregs.[97] In a murine colorectal cancer model, elevated TGF-β signaling was associated with poorly immunogenic tumors and limited response to ICB, indicating resistance.[98] In line with these findings, improved antitumor response to ICB was seen with inhibition of TGF-β in metastatic urothelial cancer.[99]

In addition to promoting angiogenesis, VEGF functions as an immunosuppressive cytokine and is associated with resistance to ICB. VEGF levels were found to be higher in anti–PD-1 therapy nonresponders compared with responders.[100] In mouse models, VEGF impeded commitment of lymphoid progenitors, reducing progression to the T-cell lineage.[101] Additionally, VEGF signaling reduces trafficking and extravasation of CTLs into the TME while it promotes infiltration of Tregs through a selective endothelium.[102] Furthermore, VEGF increases expression of inhibitory receptors, contributing to CTL exhaustion.[103] Corroborating this evidence, inhibition of VEGF was correlated with improved response to ICB in renal cell carcinoma.[104]

Antigen Presentation and Evolution of Immune Response

The evolving immune response, from initial antigen exposure to cancer cell cytotoxicity and memory T-cell formation, can be manipulated to evade antitumor immunity. The inability of T cells to proliferate and adequately diversify likely contributes to ICB resistance. Impaired priming of naive T cells through suppressed dendritic cell (DC) recruitment was associated with lack of TILs and ICB resistance in melanoma.[105,106] Deficiencies in antigen presentation have been shown to play a role in ICB resistance. Multiple studies demonstrated that downregulation of MHC class I (MHC-I) allows tumor cells to resist immune surveillance.[107,108] Loss of function of β2-microglobulin results in disruption of MHC-I folding and transport to the cell surface, thus mediating immune evasion of tumor cells.[109-111] An important study of patients with melanoma found truncating mutations in β2-microglobulin, leading to loss of MHC-I expression and acquired resistance to ICB.[112] Additionally, mutations within the T-cell receptor binding domain of MHC-I have been identified in colorectal cancer, abrogating cytotoxicity and contributing to immune escape.[113]

The IFN-γ signaling pathway mediates immune response through the JAK/STAT family of receptors and transducers. IFN-γ signaling upregulates expression of MHC-I, resulting in enhanced antigen presentation (Fig. 2A).[114] However, IFN-γ also functions within a negative-feedback loop to increase expression of PD-L1, conferring adaptive resistance to tumor cells.[115,116] In the context of PD-1 blockade, amplification of PD-L1 in Hodgkin lymphoma correlated with improved response to therapy.[117] Multiple studies have demonstrated that loss of JAK/STAT signaling results in resistance to PD-1 and CTLA-4 blockade through inability to upregulate MHC-I and PD-L1 expression.[112,118-121]

Overexpression of alternate immune checkpoints has been linked to anti–PD-1 and anti–CTLA-4 therapeutic failure. Adaptive resistance to ICB was observed secondary to compensatory upregulation of alternative immune checkpoint receptors, including T-cell immunoglobulin, mucin domain-3 protein (TIM-3), and lymphocyte-activation gene 3 (LAG-3), across multiple studies.[122-124] Alternate immune checkpoint receptors continue to be discovered (Fig. 1C), including 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).[125-127] Coexpression of multiple immune checkpoints has been associated with a severely exhausted T-cell state. Thommen et al[128] demonstrated a positive correlation between progressive T-cell exhaustion and increased coexpression of PD-1, CTLA-4, TIM-3, LAG-3, and BTLA, with subsequent resistance to ICB in NSCLC. Thus, these alternative immune checkpoint receptors may serve as potential therapeutic targets for blockade.

In addition to expression of inhibitory receptors, exhausted T cells demonstrate impaired effector function and altered transcriptional state compared with Teffs. T-cell exhaustion presents as a spectrum, with association seen between progressive loss of function and antigen persistence.[129] Chronic exposure to cognate antigen also results in elevated PD-1 expression, with subsequently impaired T-cell function.[130] Studies have shown that tumors with low or intermediate expression of PD-1 can be reinvigorated with ICB. However, high expression of PD-1 was correlated with accumulating T-cell exhaustion and poor response to therapy.[131,132] Recently, epigenetic changes were linked to T-cell exhaustion, in that exhausted cells were found to have a unique chromatin landscape that influenced transcriptional state and limited effector function.[133-135] Moreover, the type of distinct chromatin state determined if exhausted T cells could be reprogrammed after therapy to avoid terminal exhaustion.[136]

Following effector activity, a minority of T cells enter a memory phase, remaining quiescent until antigen rechallenge.[137,138] Chronic antigen exposure renders precursor memory T cells exhausted, with eventual deletion and lack of memory formation.[139,140] Given that success of ICB is highlighted by marked response durability, memory T-cell formation plays an important role in avoiding recurrence and resistance following cessation of treatment. Accordingly, patients who responded poorly to anti–PD-1 therapy were shown to harbor fewer tumor-associated memory T cells compared with responsive patients.[141]

Classic Oncologic Pathways

Through aberrations in oncogenes and tumor suppressors, oncologic signaling pathways can regulate immune

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