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Resistance Mechanisms to Anti-PD Cancer Immunotherapy

Matthew D. Vesely,[1,2] Tianxiang Zhang,[1] and Lieping Chen[1,2,3]

[1]Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut, USA; email: lieping.chen@yale.edu

[2]Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, USA

[3]Department of Medicine (Medical Oncology), Yale University School of Medicine, New Haven, Connecticut, USA

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Abstract

The transformative success of antibodies targeting the PD-1 (programmed death 1)/B7-H1 (B7 homolog 1) pathway (anti-PD therapy) has revolutionized cancer treatment. However, only a fraction of patients with solid tumors and some hematopoietic malignancies respond to anti-PD therapy, and the reason for failure in other patients is less known. By dissecting the mechanisms underlying this resistance, current studies reveal that the tumor microenvironment is a major location for resistance to occur. Furthermore, the resistance mechanisms appear to be highly heterogeneous. Here, we discuss recent human cancer data identifying mechanisms of resistance to anti-PD therapy. We review evidence for immune-based resistance mechanisms such as loss of neoantigens, defects in antigen presentation and interferon signaling, immune inhibitory molecules, and exclusion of T cells. We also review the clinical evidence for emerging mechanisms of resistance to anti-PD therapy, such as alterations in metabolism, microbiota, and epigenetics. Finally, we discuss strategies to overcome anti-PD therapy resistance and emphasize the need to develop additional immunotherapies based on the concept of normalization cancer immunotherapy.

INTRODUCTION

Fundamental understanding of immune cell regulation, particularly T cell activation and inhibition (1–10) as well as diverse mechanisms in the control of immune responses in tumor tissues or the tumor microenvironment (TME), has ushered in a new therapeutic approach: anti-PD therapy, which uses monoclonal antibodies to block the PD-1 (programmed death 1)/B7-H1 (B7 homolog 1) pathway (11–13). The clinical translation of these immunotherapeutics in the last two decades (14–16) has had remarkable success in the treatment of advanced human cancers, especially solid tumors, and immuno-oncology departments have been established across academia, pharmaceutical industry, and hospitals. The US Food and Drug Administration (FDA) has approved anti-PD therapy for more than 20 types of solid tumors. This therapy has become not only a new clinical standard but also the backbone for future combination therapy. Despite these successes, only a fraction of cancer patients benefit from this therapy, and our understanding of clinical success and failure is limited. It has become clear that highly diverse resistance mechanisms exist in the TME and may play major roles in impairing immune responses, leading to resistance to anti-PD therapy. Therefore, a careful interrogation of the TME and a comprehensive understanding of why certain immunotherapies fail are critical for future cancer immunotherapy.

Tumor Immunity in the Microenvironment

Although cancer begins as a disease of genetic mutations that transform cells and allow them to break free of cellular growth restraints (17), most cancers require much more than cancer cells to establish malignancy and metastasis. An alternative and disorganized tissue composed of cancer cells, immune cells, and stromal cells that sustain and promote cancer outgrowth is referred to as the TME. Every component of the TME may be critical for tumorigenic potential and is therefore a potential target for cancer therapy. Unequivocal evidence shows that spontaneous immune responses against cancer occur to control tumor development (18). Escape from this control is not only a hallmark of cancer (19) but perhaps the most successfully targeted aspect of the TME. Understanding the tumor immunity in the microenvironment (TIME) of cancers, therefore, is critical for designing and implementing the best immunotherapy (20–22).

Anti-PD Therapy as Normalization Cancer Immunotherapy

Recent enthusiasm for cancer immunotherapy is predominately due to the success of targeting the immunosuppressive TME with monoclonal antibodies that block immune inhibitory receptor PD-1 and its ligand B7-H1, also known as programmed death ligand 1 (PD-L1) (23). Targeting the PD-1/B7-H1 inhibitory axis, termed anti-PD therapy, has resulted in durable responses in patients for multiple distinct types of cancers (11, 24, 25). Although immune-related adverse events may limit anti-PD therapy responses, the focus of this review will be on mechanisms of resistance within the TME. In fact, among therapies for cancer, anti-PD therapy has the most favorable ratio of beneficial clinical response to systemic toxicity, and it is quickly becoming the new standard of care for a spectrum of advanced malignancies (24, 25). This is underscored by six distinct FDA-approved drugs targeting the anti-PD pathway (and four additional anti-PD drugs approved in China) for 20 cancer indications and more than 3,600 active clinical trials involving anti-PD therapy (26, 27).

The most likely reason for the success of anti-PD therapy stems from targeting a local dysfunctional immune response within the TME, rather than boosting systemic immunity, for example, with cytokine and anti-CTLA-4 (cytotoxic T lymphocyte–associated) therapy. We previously described the key differences between immunotherapies that enhance underlying

immune responses systemically, called enhancement cancer immunotherapies, and those that repair a dysfunctional immune response locally within the TME and bring immune responses into the normal range (24, 25). Anti-PD therapy is the archetypal normalization cancer immunotherapy.

Primary Versus Acquired Resistance to Anti-PD Therapy

Why patients do not respond to anti-PD therapy is one of the major questions in modern cancer therapy (28). Multiple terms have been proposed for each type of resistance to anti-PD therapy, and terminology is not consistent throughout the field. Broadly, resistance to anti-PD therapy can be classified based on clinical outcomes, primary resistance and acquired or secondary resistance, or based on biological mechanisms, including innate and adaptive resistance (29–31). In primary resistance, tumors fail to respond from the beginning of anti-PD therapy. In contrast, acquired resistance is where tumors initially respond to anti-PD therapy but then disease progresses (**Figure 1*a*,*b***). It follows that primary resistance often includes mechanisms of innate resistance and acquired or secondary resistance includes adaptive immune resistance mechanisms. Recently, the Society for Immunotherapy of Cancer (SITC) published initial recommendations from its multi-stakeholder taskforce comprising experts across academia, government, and pharmaceutical and biotechnology industries to refine clinical definitions for resistance to anti-PD therapy (32). The SITC Immunotherapy Resistance Taskforce defined resistance to anti-PD therapy as either (*a*) primary resistance or (*b*) secondary resistance based on the clinical parameters of duration of drug exposure and time to progression. In this manner, secondary resistance replaces the term acquired resistance, which has been used by others to describe both clinical and biological scenarios. Another type of resistance they discussed is disease progression after treatment discontinuation or halting therapy because of toxicity. The intent of the SITC recommendations is to develop clear guidelines for clinical trial enrollment and not to define biological mechanisms that may be involved in both primary and secondary resistance.

Adaptive immune resistance is distinct from primary and secondary resistance, which are clinical outcomes, in that it involves pathways protecting normal tissue from the detrimental effects of immune reaction that are used by tumors in response to ongoing immune attack (29, 30, 33). The focus of this review is biological mechanisms of resistance and the dysfunctional immune response within the TME that may occur in either clinical primary or secondary resistance or both.

The best-known mechanism and basis for the concept of adaptive resistance is the expression of B7-H1 within the TME in response to T cell release of IFN-γ (20). Tumors adapt to immune attack by developing new tactics. However, it is important to note that the same mechanism, such as IFN-γ release, can be critical for primary and secondary resistance to immunotherapy. In this article, we focus on the cellular and molecular processes underlying resistance to anti-PD therapy as a framework to discuss mechanisms of resistance rather than categorizing them as primary, adaptive, or acquired. Wherever possible we focus on human data and present mechanistic insight using preclinical studies only when patient data are unavailable.

MECHANISMS OF IMMUNE RESISTANCE TO ANTI-PD THERAPY

Most of the evidence on resistance mechanisms to anti-PD therapy, unsurprisingly, involves immune-mediated mechanisms. Therefore, the bulk of our discussion concentrates on immune evasion mechanisms used by cancers to thwart immune attack as well as TIME that are less amenable to anti-PD therapy. For example, tumors that lack B7-H1 are less likely to respond to anti-PD therapy (34) and likely have alternative immune escape mechanisms. In addition to these

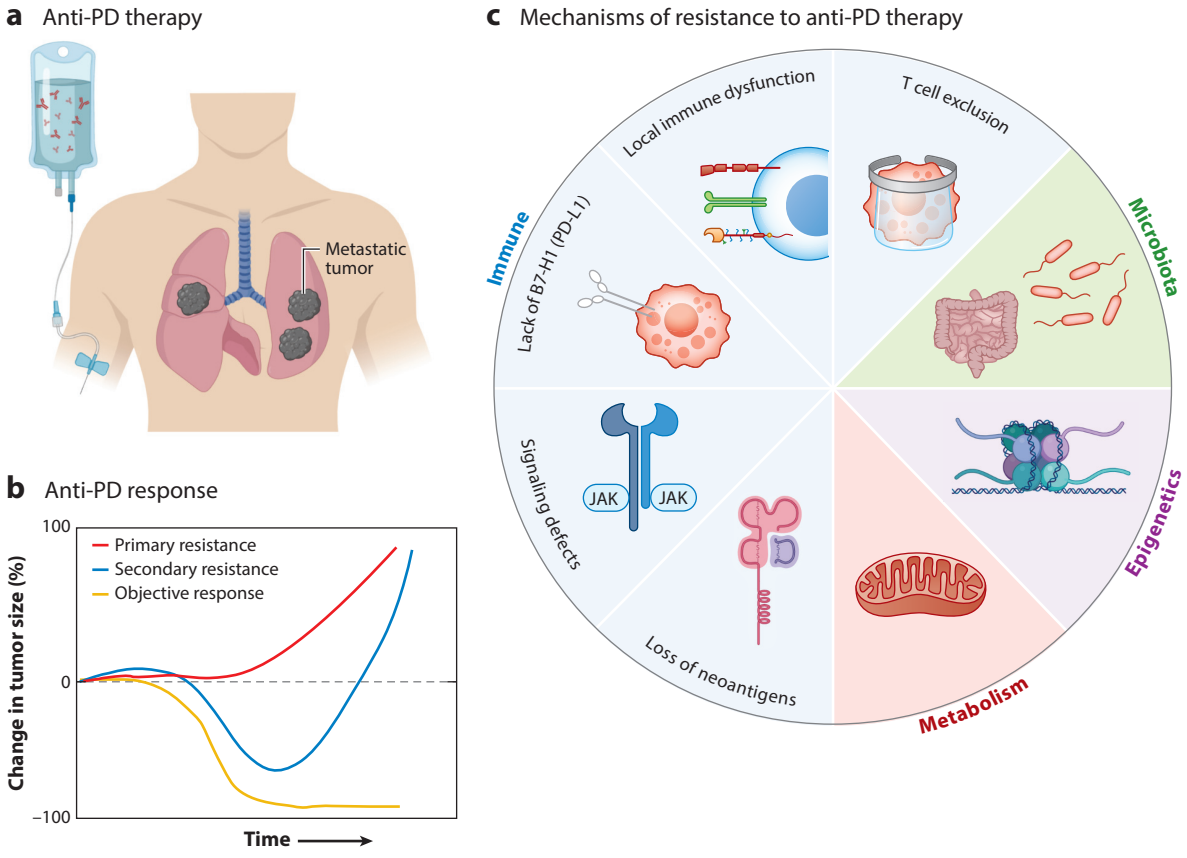


Figure 1

Anti-PD therapy resistance to cancer immunotherapy. (*a*) Patient with metastatic cancer receiving anti-PD therapy. (*b*) Clinical responses to anti-PD therapy include primary resistance (*red*), secondary resistance (*blue*), and objective response (*yellow*). In primary resistance, the tumor does not respond to anti-PD therapy. In secondary resistance, the tumor initially responds to anti-PD therapy but then grows. An objective response is when the tumor's response to anti-PD therapy provides a durable clinical benefit. (*c*) Mechanisms of resistance to anti-PD therapy include established immune-based mechanisms (*blue*) such as loss of neoantigens, defects in antigen presentation and interferon signaling, lack of B7-H1 (PD-L1), local immune dysfunction through expression of immune inhibitory molecules, and exclusion of T cells. Emerging mechanisms of resistance to anti-PD therapy include activity of the gut microbiota (*green*), epigenetics (*purple*), and alterations in metabolism (*red*). There is a lack of data from human cancer patients treated with anti-PD therapy to confirm the roles of metabolism, epigenetics, and the microbiota in resistance to anti-PD therapy. Abbreviations: B7-H1, B7 homolog 1; JAK, Janus kinase; PD-L1, programmed death ligand 1.

immune-mediated resistance mechanisms, we discuss new insights into metabolism, microbiota, and epigenetics that affect anti-PD therapy responses (**Figure 1*c***).

It is important to note that innate immune activation and stimulation of naive T cells are critical for the development of antitumor immunity (35). Costimulation is necessary after T cell receptor (TCR) recognition of tumor antigens for T cells to develop cytotoxic functions and eliminate cancer cells. Enhancement of the antitumor immune response through activating signals is termed enhancement therapy (24, 25). Several costimulatory agonist antibodies are in clinical development (36). A lack of immune priming and initiation may play a role in resistance to anti-PD therapy, but most evidence from human patients suggests that T cells within tumors or in adjacent

lymph nodes are activated and become dysfunctional (24, 25). In this review, we focus on resistance mechanisms within the TME that restrain activated T cells.

Tumor escape from immunotherapy is often due to adaptive immune resistance (29), where T cell attack (IFN-γ) results in compensatory upregulation of local evasion mechanisms (B7-H1) within the TME (20). Anti-PD therapy can overcome this local immune dysfunction for tumors expressing B7-H1 (37). However, there are many other mechanisms of immune resistance operating within the TME that limit the effectiveness of anti-PD therapy. Resistance mechanisms are generally direct or indirect. Direct mechanisms affect the PD-1/B7-H1 pathway and prevent anti-PD therapy from working properly. Examples include lack of B7-H1 expression, loss of the interferon signaling pathway, and T cell exclusion in the TME. Therefore, these direct mechanisms are also known as target-missing resistance (29). The indirect mechanisms are not specific for anti-PD therapy and are utilized by cancer to resist possibly all types of immunotherapy, such as loss of antigen and lack of antigen presentation. Here we discuss the clinical evidence for immune-mediated resistance to anti-PD therapy, which is the most established and best-studied category of resistance (**Figure 2**). It is also important to note that because TMEs of advanced cancers are very heterogeneous, many of the mechanisms described here are not present in all tumors. In fact, a particular mechanism may operate in a fraction of patients at a particular time period.

Lack of B7-H1 Expression in the Tumor Microenvironment

The primary action of anti-PD therapy is blockade of the PD-1/B7-H1 pathway by specific monoclonal antibodies to either B7-H1 or PD-1 in the TME (13). Therefore, lack of expression of either B7-H1 or PD-1 in the TME represents a direct resistance mechanism. Initially based on the observation that melanomas express distinct patterns of T cell infiltration and B7-H1 (20), a TIME classification scheme featuring these two parameters was proposed (20, 21). It groups cancers into four types: Type I cancers have no tumor-infiltrating T cells or B7-H1, type II cancers have both T cells and B7-H1, type III cancers have T cells but not B7-H1, and type IV cancers have B7-H1 but not T cells (21, 22, 29, 38) (**Figure 3**). Therefore, the type I, III, and IV cancers, which constitute more than 50% of all cancer cases, would not be expected to respond to anti-PD therapy because they lack one or both key target proteins. For example, according to accumulated data on non–small cell lung cancer (NSCLC), 57% of these cancers lack tumor-infiltrating T cells or tumor-infiltrating lymphocytes (TILs) (45% type I and 12% type IV) (39, 40). In contrast, 26% of NSCLCs have TILs but lack B7-H1 (type III), leaving only 17% of NSCLCs predicted to respond to anti-PD therapy due to the presence of both TILs and B7-H1 (type II) (**Figure 3**). This simple TIME classification method explains why a large portion of cancers do not respond to anti-PD therapy. Similar classifications of TIME have been proposed (41–43) to inform current and future therapeutic strategies (44, 45), and accumulated data across several independent studies reveal that more than half of cancers do not express B7-H1 and/or do not have infiltrating T cells and are associated with poor response to anti-PD therapy (29).

Application of these parameters in the clinic to predict the outcome of anti-PD therapy, however, is not a simple task. B7-H1 protein detection is largely dependent on clinical biopsy of tumor tissues, with small samples and a single time point. The small biopsy specimens may not be representative, because B7-H1 expression is not evenly distributed in tumor tissues and is often adjacent to the IFN-γ–producing TILs (20). The dynamic immune response occurring within the TME and the expression of B7-H1 may change from time to time. This explains why some patients with low or no B7-H1 detected in their tumor tissue biopsies responded to anti-PD therapy (34). In addition to B7-H1 expression, effector $CD8^+$ T cells are also critical for anti-PD therapy (37). It

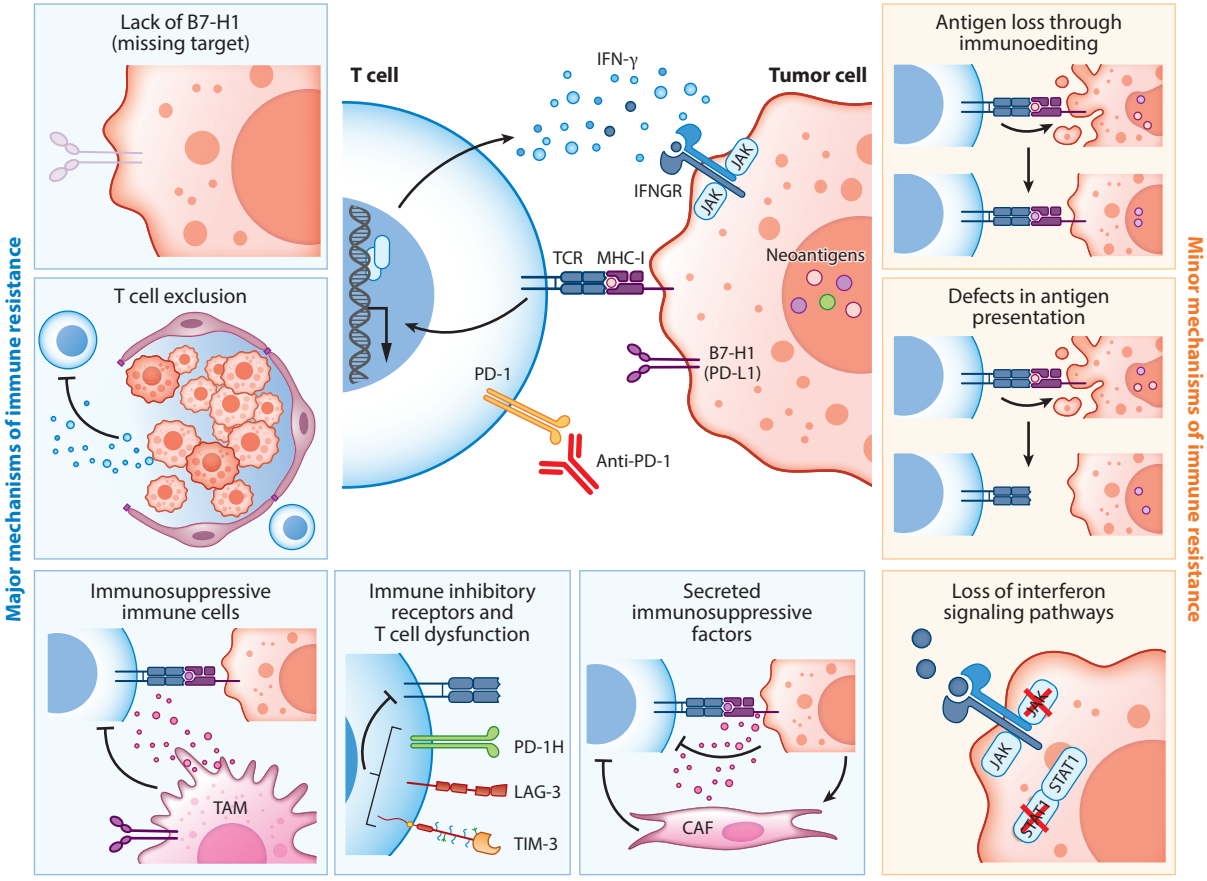


Figure 2

Mechanisms of immune-mediated resistance to anti-PD therapy. (*Center*) An effective antitumor immune response by a T cell (*blue*) recognizing and attacking a cancer cell (*red*). (*Left*, *bottom*; *blue boxes*) The major mechanisms of immune resistance to anti-PD therapy, which help explain primary and secondary resistance observed in many human cancer patients, include the lack of B7-H1 (also known as PD-L1), or missing target resistance; T cell exclusion; immunosuppressive immune cells within the tumor microenvironment; and T cell dysfunction through other immune inhibitory molecules such as PD-1H (VISTA), LAG-3, and TIM-3 as well as secreted immunosuppressive factors from cancer cells and/or cancer-associated fibroblasts. (*Right*, *orange boxes*) In contrast, minor mechanisms of immune resistance to anti-PD therapy are found in small numbers of patients, and the details of the involvement of these mechanisms in primary and secondary resistance remain unknown. However, they may be critical resistance mechanisms for some cancers. They include antigen loss through immunoediting, defects in antigen presentation, and loss of interferon signaling pathways. Abbreviations: B7-H1, B7 homolog 1; CAF, cancer-associated fibroblast; IFNGR, IFN-γ receptor; JAK, Janus kinase; LAG-3, lymphocyte-activation gene 3; PD-1, programmed death 1; PD-1H, PD-1 homolog; PD-L1, programmed death ligand 1; STAT1, signal transducer and activator of transcription 1; TAM, tumor-associated macrophage; TCR, T cell receptor; TIM-3, T cell immunoglobulin and mucin domain–containing molecule 3; VISTA, V domain immunoglobulin suppressor of T cell activation.

is particularly interesting that type III tumors have TILs present but lack B7-H1 expression. This finding indicates that TILs in the TME do not produce IFN-γ and may be dysfunctional.

T Cell Exclusion

PD-1 is mainly found on lymphoid cells, especially T cells. Absence of T cells in the TME would represent another target-missing resistance mechanism. As mentioned above, more than 50% of

advanced human cancers do not have significant TILs in the TME (cold tumors), indicating there are also factors that prevent the infiltration of T cells. Overcoming T cell exclusion and increasing T cell infiltration into tumors is a major challenge to improve clinical outcomes for anti-PD therapy. Several cytokines such as TGF-β and VEGF are elevated in the TME, and when they are blocked in preclinical animal models, T cell infiltration increases and enhances the efficacy of anti-PD therapy (46–48). Preclinical and clinical studies have implicated both tumor cells and stromal cells within the TME as contributing to T cell exclusion (49). Several studies suggest multiple mechanisms that prevent T cells from infiltrating tumors (50–52), and blockade of these pathways in human cancer patients is yet to be tested.

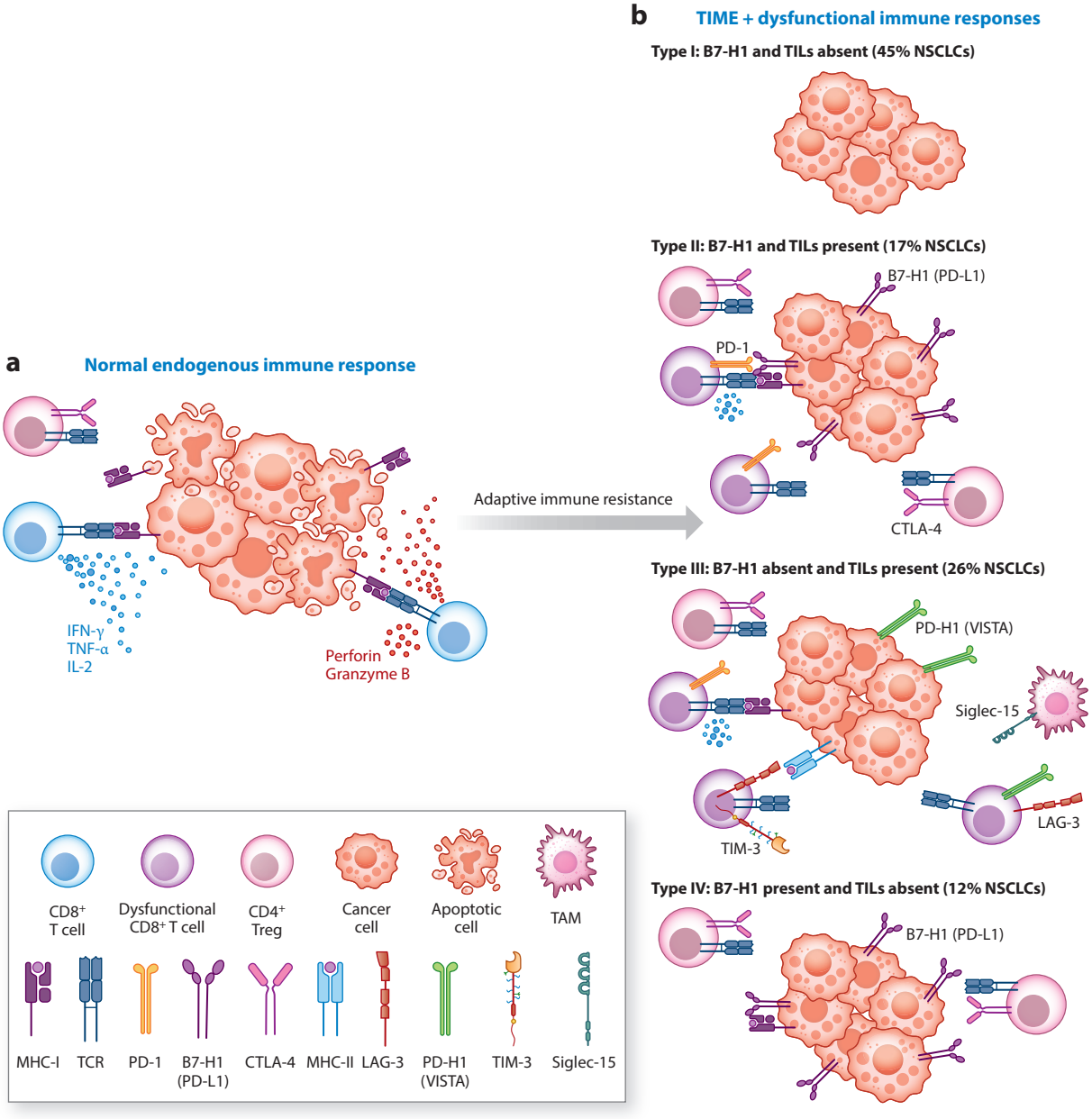
The oncogenic pathways within cancer cells can often promote evasion of antitumor immune responses by excluding T cells from cancers (53). For example, phosphatase and tensin homolog (PTEN) is associated with T cell infiltration of tumors; regions of melanoma cells that express PTEN within a tumor colocalize with more T cell infiltration. Melanoma patients whose tumor cells lose *PTEN* expression have worse clinical outcomes (54). In a small number of cancer patients that were initially responding to anti-PD therapy and subsequently developed progressive disease, loss of *PTEN* expression was observed in the tumor at the time of resistance (55, 56). In addition to PTEN, the WNT-β-catenin pathway is also associated with a lack of T cell infiltration into tumors (57). In one study, a melanoma patient who initially responded to anti-PD therapy developed metastases that had newly elevated β-catenin expression and lost T cell infiltration (56). Single-cell RNA sequencing of melanoma cells from a cohort of 33 patients identified a resistance transcriptional program that is associated with T cell exclusion and that predicted clinical responses to anti-PD therapy in an independent cohort of 112 melanoma patients (58). The resistance and exclusion programs were elevated in immunotherapy-resistant lesions, suggesting that this transcriptional program is associated with anti-PD therapy resistance. One class of genes upregulated in melanomas that excluded T cells encoded cyclin-dependent kinases, under the control of oncogenes. Consistent with this, CDK4/6 inhibitors enhanced T cell infiltration and immunotherapy in a preclinical melanoma model (58).

Loss of Interferon Signaling Pathways

Interferons are critical for the antitumor immune response and are perhaps the most critical cytokines involved in cancer elimination, owing to their pleiotropic effects of reducing tumor cell proliferation; increasing tumor cell antigen processing and presentation; activating antigen-presenting cells, such as dendritic cells and macrophages; and ultimately increasing the functions of both innate and adaptive effector immune cells, including tumor cell cytotoxicity (59). Both tumor cell interferon signaling and immune cell interferon signaling are critical for effective antitumor immunity (60–62). Type I interferons (IFN-α/β) and type II interferon (IFN-γ) utilize the downstream signaling proteins Janus kinase (JAK) and signal transducer and activator of transcription (STAT) to effect intracellular changes (63, 64). Despite all these positive functions of interferons, IFN-γ is a major cytokine in upregulating and maintaining B7-H1 expression in the TME (6) by both transcriptional and posttranscriptional control (65). Given that upregulated B7-H1 was able to engage PD-1 on T cells to induce dysfunction, interferons in the TME may be a double-edged sword for tumor immunity (6). This finding explains how tumor cells can adapt in the TME. Therefore, a defect in an interferon signaling pathway could nullify both positive and negative effects and possibly render resistance to anti-PD therapy.

A subset of human cancers have mutations affecting interferon signaling, including in the downstream signaling molecule JAK. For example, human prostate cancer cell line LNCaP lacks JAK1 expression, rendering it insensitive to interferons (66). In a study by Ribas and colleagues (67) that identified *B2M* mutations in tumor cells, two patients with metastatic melanoma that initially

responded to anti-PD therapy, pembrolizumab, but subsequently progressed were found to have loss-of-function mutations in *JAK1* and *JAK2*. The *JAK2* mutation resulted in a loss of JAK2 protein expression in generated cell lines and a subsequent lack of response to IFN-γ and inability to upregulate B7-H1 and MHC-I. A subsequent study identified *JAK1/2* loss-of-function mutations in 1 of 23 patients with melanoma and 1 of 16 patients with colon cancer treated with anti-PD therapy (68). The functional consequence of these loss-of-function JAKs is an inability to respond to



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

TIME and anti-PD therapy missing target resistance. (*a*) Within the tumor microenvironment, T cells recognize and destroy cancer cells through various mechanisms, including IFN-γ, TNF-α, perforin, and granzyme B activities. (*b*) Over time, tumors develop immune escape mechanisms, such as increased expression of B7-H1 (PD-L1), in a process called adaptive immune resistance that results in a dysfunctional immune response. Clinically apparent tumors have local dysfunctional immune responses, and the TIME can be characterized using two parameters: T cell infiltration and B7-H1 (PD-L1) expression. With this approach, cancers can be grouped into four types: cancers without tumor infiltrating T cells or B7-H1 (type I), cancers with both tumor-infiltrating T cells and B7-H1 (type II), cancers with T cells but not B7-H1 (type III), and cancers with B7-H1 but not T cells (type IV). For example, 57% of NSCLCs lack T cells (45% are type I and 12% type IV) (39, 40). In contrast, 26% of NSCLCs have TILs but lack B7-H1 (type III), leaving only 17% of NSCLCs that are predicted to respond to anti-PD therapy due to the presence of both TILs and B7-H1 (type II). Type III tumors often have other mechanisms of immune-mediated resistance, including immunosuppressive immune cells and upregulation of other immune inhibitory molecules, such as PD-1H (VISTA), TIM-3, LAG-3, and Siglec-15. Abbreviations: B7-H1, B7 homolog 1; CTLA-4, cytotoxic T lymphocyte–associated; LAG-3, lymphocyte-activation gene 3; NSCLC, non–small cell lung cancer; PD-1, programmed death 1; PD-1H, PD-1 homolog; PD-L1, programmed death ligand 1; TAM, tumor-associated macrophage; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; TIM-3, T cell immunoglobulin and mucin domain–containing molecule 3; TIME, tumor immune microenvironment; Treg, regulatory T cell; VISTA, V domain immunoglobulin suppressor of T cell activation.

IFN-γ and regulate B7-H1 expression. In an analysis of 905 cancer cell lines from the Cancer Cell Line Encyclopedia, approximately 0.7% of human cancer cell lines had *JAK1/2* mutations predicted to prevent responsiveness to interferons (68). Analysis of skin cutaneous melanomas, breast invasive carcinomas, prostate adenocarcinomas, lung adenocarcinomas and colorectal adenocarcinomas available from The Cancer Genome Atlas (TCGA) reveals that 6–12% of these cancers harbor *JAK1/2* mutations (68). A subset of human melanoma cell lines established from metastatic lesions showed defects in genes regulating IFN-γ signaling (69). Among the 46 cell lines submitted for exome sequencing, 5 (11%) had mutations in *JAK1* ($n = 3$), *JAK2* ($n = 1$), or *STAT1* ($n = 1$), rendering these cell lines insensitive to T cell–mediated killing. The impact of these mutations on survival remains largely unknown, although *JAK1/2* mutations predicted to result in loss of function and reduce responsiveness to interferons have been associated with worse survival (68). More work is needed to determine how common loss of interferon signaling contributes to anti-PD therapy resistance and whether feasible interventions to overcome this loss can be implemented.

Antigen Loss Through Immunoediting

The dual roles of the immune system to both eliminate tumor cells and promote tumor growth are often referred to as cancer immunoediting (18, 70). Networks of immune cells, tumor cells, and stromal cells within the TME result in one of three outcomes for the tumor—elimination, equilibrium, or escape (71). In preclinical models, T cell–mediated detection of tumor-specific antigens, such as neoantigens, is critical for tumor cell destruction (72) and possibly also for response to anti-PD therapy (73–75). Recognition of neoantigens is sometimes referred to as the final common pathway of cancer immunotherapy (76), as multiple distinct types of immunotherapy, including anti-PD therapy, require recognition and subsequent destruction of tumor cells. A higher tumor mutational burden is hypothesized to generate more neoantigens and has been associated with more tumor-infiltrating T cells, higher diversity of TCRs (77, 78), and improved outcome of T cell–directed immunotherapies in some studies (79, 80).

In human cancers, the predicted neoantigen burden correlates with survival and response to anti-PD therapy (78, 80). Recognition of these neoantigens by effector $CD4^+$ or $CD8^+$ T cells has also been demonstrated (81, 82). Notably, anti-PD therapy can enhance neoantigen T cell reactivity (80). Intratumoral, neoantigen-specific T cell dynamics in a patient with melanoma demonstrated that loss of tumor cell neoantigen expression can occur over time, consistent with cancer immunoediting (82). Therefore, immunoselection through an active antitumor response can result in the outgrowth of neoantigen-loss variants and tumor immune escape. This mechanism

of immune escape through neoantigen loss can occur early in untreated cancers, revealing a strong selective pressure by the immune system (83). This evolution of the neoantigen landscape can also occur during anti-PD therapy, resulting in therapeutic resistance (84). In a study of NSCLC, matched pretreatment and posttreatment biopsies from patients with tumors resistant to anti-PD therapy were analyzed for neoantigen changes through whole-exome sequencing and TCR clonotyping (84). Of the 42 NSCLC patients, 4 were found to develop resistance to anti-PD therapy through loss of putative mutation-associated neoantigens as opposed to defects in antigen-processing and -presentation machinery. Predicted neoantigen peptides were sequenced and were recognized by the same patient's T cells, demonstrating that endogenous T cell responses to neoantigens could occur prior to anti-PD therapy but subsequently be lost after anti-PD therapy through cancer immunoediting (84). In another cohort of 88 early-stage, untreated NSCLC patients, neoantigen depletion through copy-number loss was identified in 43 patients (83). An epigenetic mechanism of cancer immunoediting was identified in the TRACERx RNA-seq cohort, where epigenetic modification through promotor hypermethylation and subsequent neoantigen silencing were found in 28 of 64 NSCLC patients (83).

Defects in Tumor Antigen Presentation

Tumor cells may have defects in antigen-processing and -presentation machinery that hinder the immune system's ability to detect neoantigens present within cancer cells. Although this mechanism has been widely demonstrated in experimental conditions with preclinical models, it has been difficult to determine how frequently this occurs in response to anti-PD therapy. Mutations in MHC-I have been found in human cancer (85). Using a computational tool to identify loss of heterozygosity in human leukocyte antigen (HLA), McGranahan et al. (86) found that 40% (36/90) of NSCLCs had HLA loss through loss of heterozygosity, resulting in loss of neoantigen binding and immune escape. In a cohort of untreated NSCLC patients, HLA loss of heterozygosity was observed in 56% of lung adenocarcinomas and 78% of lung squamous cell carcinomas (83). It is unknown whether HLA loss of heterozygosity prevents presentation of tumor antigens during anti-PD therapy. One recent study identified a patient with metastatic melanoma that initially responded to anti-PD therapy, pembrolizumab, but subsequently progressed. Tumor cell exome sequencing from longitudinal biopsies obtained prior to and during anti-PD therapy revealed a truncating mutation in the β_2-microglobulin (β_2m) gene (*B2M*) (67). This mutation results in the absence of B2M, which is necessary for stabilization of HLA class I. Thus, MHC-I may be unstable and unable to present neoantigens to TILs. Gettinger and colleagues (87) analyzed 14 lung cancers resistant to anti-PD therapy; 1 tumor had homozygous loss of *B2M*, resulting in absence of HLA class I cell surface expression, and 2 others had downregulation of B2M. In another study on metastatic melanoma, longitudinal tumor biopsies were performed for 17 patients that progressed on immunotherapy. Among these 17 patients, 5 (29.4%) patients' tumors exhibited defects in *B2M* (88). In contrast, no *B2M* alterations were detected in patients that responded to immunotherapy. One remarkable aspect of anti-PD therapy is that it is the first cancer therapy to be approved for a molecular signature rather than a specific cancer type. In cancers with loss of function in the mismatch repair pathway, effective immune control can be achieved by anti-PD therapy (89). Two patients with mismatch repair–deficient tumors that developed resistance to anti-PD therapy were found to have mutations in *B2M* (89). Taken together, studies of independent cohorts have identified defects in antigen presentation both prior to and during anti-PD therapy. Despite these remarkable cases across distinct cancer types, it is unclear how common this mechanism of resistance is. This may reflect tumor sample acquisition and underscores the need for longitudinal biopsies of tumors in patients undergoing

immunotherapy. In addition, B2M is expressed ubiquitously, and high levels of soluble B2M can be detected in sera of healthy people and cancer patients (90). In theory, this soluble B2M protein might stabilize HLA to compensate for the loss of B2M in tumor cells. Therefore, it is yet to be determined whether loss of B2M results in loss of tumor antigen presentation.

Immunosuppressive Cells

Immunosuppressive cells such as regulatory T cells (Tregs) (91), myeloid-derived suppressor cells (MDSCs) (92), and tumor-associated macrophages (TAMs) (93) impair the antitumor immune response in preclinical cancer models. Infiltration of Tregs into the TME has been demonstrated in a wide variety of cancer types (94–98) and negatively impacts clinical outcomes (94, 98). The effect of anti-CTLA-4 therapy on the modulation of immune responses may be due to its targeting Tregs, at least partially (99–104). Much less is known about how Tregs affect responses to anti-PD therapy. Although associations between changes in Tregs and response to anti-PD therapy have been observed (105), specific changes within the TME are largely unexplored. This may be because most changes in response to anti-PD therapy occur in the TME rather than in peripheral lymphoid organs.

MDSCs are heterogeneous myeloid cells recruited to the TME that have immunosuppressive functions (92). Earlier studies identified MDSCs as a negative prognostic factor (106), but more recent data across multiple cancer types are not conclusive in this regard (107). This may be due to the analysis of different cell subsets: Circulating MDCSs and tumor-infiltrating MDSCs likely have distinct functions. Furthermore, different subsets of MDSCs may impact survival differently (108). Multiple preclinical studies have demonstrated that targeting MDSCs can augment immunotherapy, including anti-PD therapy, and current clinical trials are exploring this combination (109). These studies in human patients may help determine whether MDSCs are critical mediators of anti-PD therapy resistance seen in the clinic.

Tumor-associated macrophages (TAMs) are another group of suppressive myeloid cells attracted to the TME with similar mechanisms of immunosuppression. Similar to MDSCs, TAMs have been implicated as a predictor of patient outcomes, and targeting these cells is an area of intense clinical research (93). For example, TREM2 is expressed by tumor-infiltrating macrophages and promotes tumor growth during anti-PD therapy in preclinical studies, and TREM2 expression correlates with poor prognosis in human cancers (110). Reprogramming myeloid cells within the TME that contribute to immunotherapy resistance has been effective in preclinical models, providing a foundation for targeting myeloid cells in human cancers (111, 112). It is difficult to predict whether targeting suppressive myeloid cells to overcome resistance to anti-PD therapy will be successful in human cancer patients. Recently, targeting the immunosuppressive molecule indoleamine 2,3-dioxygenase 1 (IDO1), which is expressed by suppressive myeloid cells, did not provide additional benefit in melanoma patients when combined with anti-PD therapy (113). These immunosuppressive cells may have important prognostic value, as they reflect active immune evasion mechanisms occurring in the TME, but there are not enough therapeutic data from human cancer patients at this time to determine that these cells are a key mechanism of anti-PD therapy resistance. Future studies may ultimately enhance the efficacy of anti-PD therapy by identifying and targeting a particular myeloid cell subset or specific inhibitory molecule expressed by myeloid cells that broadly neutralizes the effect of anti-PD therapy in cancer patients.

Coinhibitory Molecules in the Tumor Microenvironment

Expression of coinhibitory molecules within the TME is a fundamental mechanism of tumor immune escape. Effector T cells are inhibited and become dysfunctional through engagement

of inhibitory receptor-ligand interactions. Successful targeting of the PD-1/B7-H1 coinhibitory axis demonstrates the powerful approach of blocking local dysfunctional T cells, allowing for T cell reinvigoration and subsequent tumor elimination or control. Multiple immune coinhibitory pathways discovered in the last decade have been targeted for cancer immunotherapy in preclinical models (114). Here we discuss those that may contribute to resistance to anti-PD therapy in cancer patients.

The lymphocyte-activation gene 3 protein (LAG-3) is expressed on T cells and delivers a coinhibitory signal to T cells, assuming this occurs when it engages with its canonical ligand MHC-II (115, 116). Dysfunctional T cells within the TME express higher levels of LAG-3, and several blocking antibodies are in clinical development for cancer immunotherapy (117–119). Gettinger et al. (87) biopsied anti-PD therapy–resistant NSCLC tumors before and after immunotherapy and found that LAG-3 expression had increased on TILs in 5 of the 8 tumors after immunotherapy. In addition, 3 out of 8 cases showed increase in T cell immunoglobulin and mucin domain–containing molecule 3 (TIM-3). There was also an upregulation of other immune inhibitory molecules such as T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), but these changes did not reach statistical significance. This suggests that in some patients with resistance to anti-PD therapy, there is compensatory increase in LAG-3 expression, and to a lesser extent TIM-3 expression, during treatment. Another study demonstrated that two patients that developed resistance on anti-PD therapy had upregulation of TIM-3, but not LAG-3, on tumor-infiltrating T cells (120). It is difficult to draw definitive conclusions from a small cohort about whether the upregulation of LAG-3 or TIM-3 was responsible for resistance to anti-PD therapy. Moreover, some resistant tumors in this cohort showed decreased expression of these inhibitory receptors after anti-PD therapy, implicating other mechanisms of escape. Recent studies reveal new perspectives for targeting the LAG-3 pathway by identifying fibrinogen-like 1 (FGL-1) protein as a major coinhibitory ligand for LAG-3 (121). FGL-1 is a soluble protein normally secreted at a low level by the liver, but its expression is highly upregulated in many solid tumors (121). The relative contribution of these ligands to LAG-3-mediated coinhibition and whether or not the blockade of one or both ligands will be required to maximize the effect remain unknown. Interestingly, FGL-1 binds a distinct molecular pocket on LAG-3 from MHC-II, implying that previously developed monoclonal antibodies targeting LAG-3 may have to be reevaluated for a complete blockade of the LAG-3 coinhibitory signal.

Another potential coinhibitory molecule involved in resistance is programmed death 1 homolog (PD-1H), or V domain immunoglobulin suppressor of T cell activation (VISTA), which is a member of the CD28 family (122–124). In contrast to inducible PD-1, CTLA-4, and LAG-3, PD-1H is constitutively expressed on multiple immune cells, including T cells and myeloid cell subsets. A recent study demonstrated its interaction with P-selectin glycoprotein ligand 1 (PSGL-1) to transmit a coinhibitory signal to T cells in an acidic environment, implying that this interaction has a role in the TME (125). Blockade of PD-1H in preclinical models is effective against cancer (126), and monoclonal antibodies are in clinical development for cancer immunotherapy (119). In a study of melanoma patients that were resistant to anti-PD therapy, ten matched pretreatment and progressive disease biopsies were analyzed for VISTA expression (127). Increased expression of VISTA was found in seven of the ten cases (70%), suggesting that VISTA may be a mechanism of resistance to anti-PD therapy.

In addition to coinhibitory molecules that are upregulated in response to anti-PD therapy, there may be tumors that do not express B7-H1 and instead express other inhibitory pathways. Tumors that lack B7-H1 would be less amendable to anti-PD therapy and have missing-target resistance, like the type I and III TIME tumors described above (29). Type III TIME tumors

lack B7-H1 but have infiltrated T cells, which are most likely rendered dysfunctional by other inhibitory pathways. A novel coinhibitory molecule that has been described recently, Siglec-15, is expressed in tumors across multiple cancer types that mostly lack B7-H1 (128). Siglec-15 is expressed on tumor-infiltrating myeloid cells, and blockade of Siglec-15 promotes antitumor immune responses. Another study has validated the finding that expression of Siglec-15 varies across tumor types and is associated with a poor prognosis of lung adenocarcinoma (129). With the phase 1 trials showing a good safety profile and encouraging clinical responses in lung cancer, head-and-neck cancer, and triple-negative breast cancer, a phase 2 clinical trial is currently testing the effectiveness of an anti-Siglec-15 monoclonal antibody for the treatment of advanced cancers (ClinicalTrials.gov identifier NCT03665285).

Secreted Immunosuppressive Factors

An immunosuppressive TME is partially achieved through the secretion of factors from tumor cells, immune cells, and stromal cells that limit antitumor immunity. Many cytokines and secreted immunosuppressive factors, including IL-8, have inhibited antitumor immunity in preclinical models and some human cancers (130). Recently, a melanoma-secreted factor was identified that promotes immune evasion and indicates poor patient prognosis and resistance to anti-PD therapy (131). Midkine is secreted by melanoma cells, and tumors with high midkine-associated expression profiles are enriched with TAMs and Tregs and are associated with poor prognosis (131). In a cohort of melanoma patients, those with higher midkine-associated expression were less likely to respond to anti-PD therapy. Tumor cells also express cytokines, such as transforming growth factor β (TGF-β), which has pleiotropic effects on cells within the TME (132). Molecular classifications of cancers with elevated TGF-β signaling are associated with poor prognosis and poor response to anti-PD therapy (133, 134), especially in cancer-associated fibroblasts. TGF-β signaling in stromal cells is also associated with less T cell infiltration and likely promotes T cell exclusion (135). Currently, multiple clinical trials targeting TGF-β in combination with anti-PD therapy are in development (132), but no results are available yet.

Another immunosuppressive molecule secreted in the TME is adenosine, which exerts local suppressive effects through multiple mechanisms. Human cancers with elevated adenosinergic pathways are associated with poor prognosis (136), and targeting this pathway has shown promise in preclinical studies. Combination clinical trials of anti-PD therapy for cancer patients are underway (137). Similar to adenosine, IDO1 is a small molecule increased in the TME that has pleiotropic effects and dampens antitumor immunity. However, dual blockade of IDO1 and PD-1 did not show additional benefit in melanoma patients (113). At this time, there is little evidence from cancer patients that secreted immunosuppressive molecules mediate resistance to anti-PD therapy.

METABOLISM AND ANTI-PD THERAPY RESISTANCE

Nearly a century ago, Otto Warburg demonstrated that cancer cells have a distinct cellular metabolism when compared to normal cells. We now appreciate that the cellular metabolism of both cancer cells (138, 139) and immune cells (immunometabolism) (140, 141) contributes to cancer progression and metastasis (**Figure 4*a***). Nevertheless, very little is known about the role of metabolism in resistance to anti-PD therapy in humans. Most evidence for the importance of cancer cell metabolism and immunometabolism during the development of the antitumor immune response comes from preclinical studies. How cancer cell metabolism contributes to an immunosuppressive TME through nutrient competition, hypoxia, acidity, and immune modulating

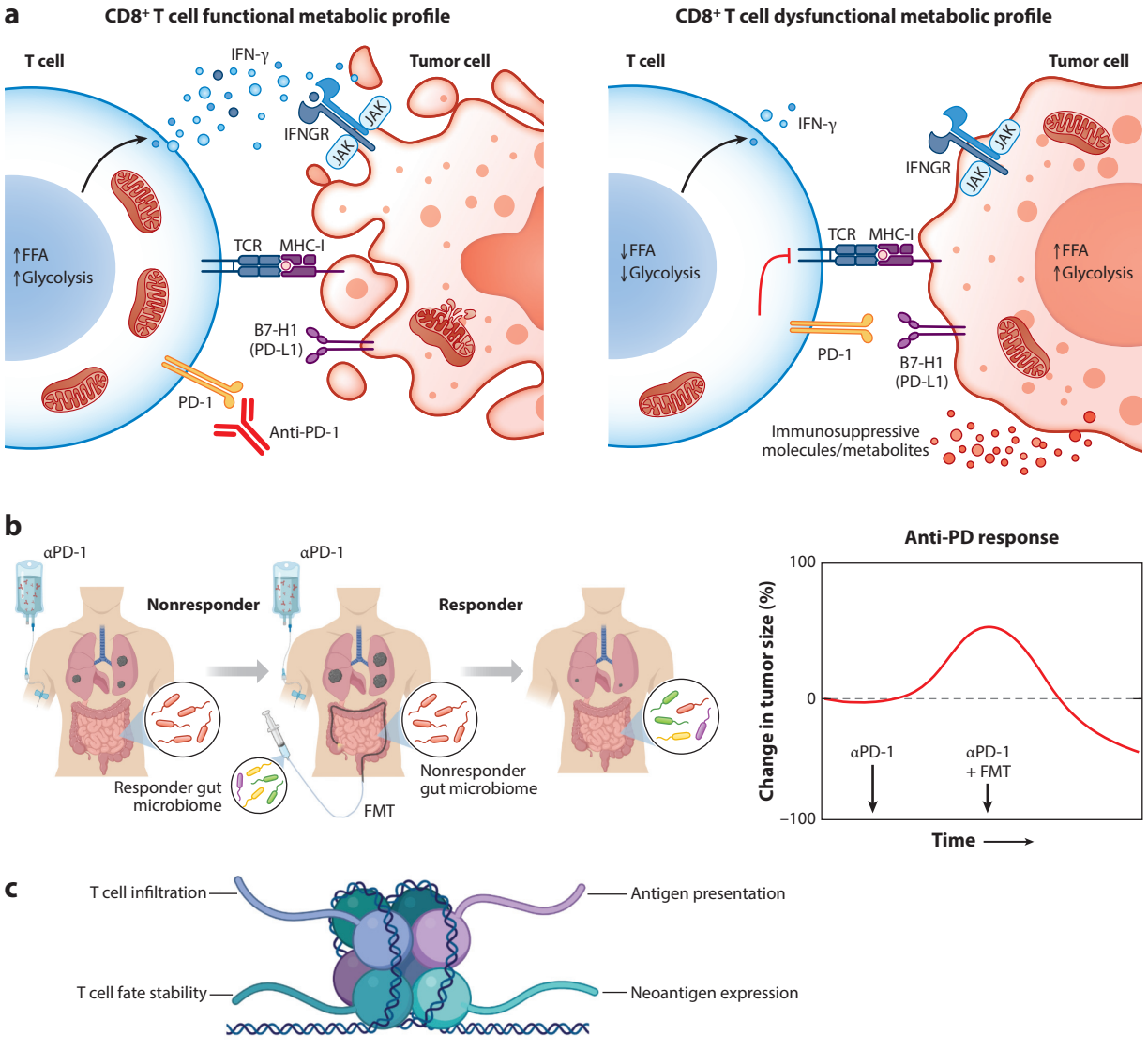


Figure 4

New and emerging mechanisms of anti-PD therapy resistance. These possible mechanisms of resistance to anti-PD therapy are proposed based on preclinical models. Due to lack of extensive clinical data, we refer to these as emerging mechanisms. We await further data from human cancer patients treated with anti-PD therapy to substantiate these mechanisms. (*a*) Metabolism is a fundamental process for both immune cells and cancer cells. Profiling of $CD8^+$ T cells shows that the metabolism of dysfunctional T cells is distinct from that of $CD8^+$ T cells that can kill cancer cells. For example, dysfunctional T cells have reduced glycolysis and free fatty acid metabolism compared to functional T cells. (*b*) An exciting emerging mechanism of resistance to anti-PD therapy is activity of the gut microbiota. Recently, two phase 1 clinical trials were conducted with a total of 25 melanoma patients who were resistant to anti-PD therapy. They received fecal microbiota transplants derived from the microbiome of responders prior to reintroduction of anti-PD therapy (184, 185). Of the 25 patients, 9 responded to anti-PD therapy with fecal microbiota transplantation. (*c*) Epigenetics is a fundamental cellular process that may affect anti-PD therapy by modulating the expression of genes involved in T cell infiltration, T cell fate, antigen presentation, and expression of neoantigens. Abbreviations: B7-H1, B7 homolog 1; FFA, free fatty acid; FMT, fecal microbiota transplantation; IFNGR, IFN-γ receptor; JAK, Janus kinase; PD-1, programmed death 1; PD-L1; programmed death ligand 1; TCR, T cell receptor.

metabolites has been reviewed elsewhere (139, 142). In addition, there are other excellent reviews on functional and metabolic phenotypes of immune cells within the TME (141). Here, we briefly discuss the evidence of metabolic reprogramming by anti-PD therapy in preclinical cancer models and emerging evidence that metabolism may be a mechanism of resistance to anti-PD therapy. Cellular metabolism regulates fundamental T cell functions, such as T cell costimulation (143), T cell activation (144), production of IFN-γ through aerobic glycolysis (145), and determination of memory T cell fate through mitochondrial fusion (146). Multiple studies have demonstrated that metabolic reprogramming can boost antitumor immune responses and enhance anti-PD therapy (147–152). Within the TME, tumor cells and T cells compete for resources, and this metabolic competition can promote cancer progression (153). In one study, anti-PD therapy resulted in T cells regaining glycolytic capacity and effector function to eradicate established cancers, suggesting that T cell hyporesponsiveness within the TME may be due to metabolic restriction (153). Moreover, B7-H1 was unexpectedly found to regulate glycolytic metabolism of tumor cells, opening another line of investigation into whether anti-PD therapy affects cancer cell metabolism (153).

Advances in technological approaches and algorithms are allowing more robust investigations into the metabolic landscape of human cancers (154, 155). Novel single-cell approaches to profile metabolic phenotypes of T cells in human colorectal cancer revealed that T cells expanding within tumors are a metabolically distinct subset as compared to excluded T cells at the tumor-immune boundary (155). Recently, one study found that prolyl hydroxylase-3 loss is associated with reduced $CD8^+$ T cell function in human cancers from the TCGA database (156).

Several recent studies in preclinical models indicate that abnormal lipid metabolism may be important in resistance to anti-PD therapy. Cholesterol is critical for T cell proliferation and activation because it regulates TCR clustering and lipid rafts (157). Inhibiting ACAT1, a cholesterol esterification enzyme, potentiates $CD8^+$ T cell functions and improves anti-PD therapy in mouse tumor models (149). In addition, Ma et al. (158) showed that TILs acquired cholesterol when entering the TME, leading to upregulation of the coinhibitory molecules PD-1, LAG-3, and TIM-3, and displayed an exhausted phenotype in preclinical tumor models. Increased cholesterol in T cells could upregulate CD36, leading to loss of function by lipid peroxidation and ferroptosis (159). A recent study showed that methionine metabolism in $CD8^+$ T cells could be impaired, resulting in low expression of STAT5 and impaired T cell immunity (160). This study also showed that methionine transporter *SLC43A2* was more highly expressed in cancers than matched normal tissues from the TCGA database and negatively correlated with *CD8* and *IFNG* transcripts in the same cancers. Infiltrating T cells from melanoma patients that had high levels of *SLC43A* transcripts had lower expression of effector genes through histone modifications and were associated with reduced T cell immune responses (160). Thus, abnormal metabolism may contribute to epigenetic alterations that contribute to T cell dysfunction within the TME.

Despite these promising studies, there does not appear to be any direct evidence that metabolism pathways in humans can differentiate responders and nonresponders to anti-PD therapy. Therefore, we cannot definitively identify metabolism as a mechanism of resistance to anti-PD therapy. This may change with future studies. It is unclear whether targeting metabolic pathways in human patients will improve T cell function and promote antitumor immune responses, as has been shown only in the preclinical models. In fact, the most promising metabolic therapy targeting IDO1 failed a phase 3 clinical trial (113), even when combined with anti-PD therapy, despite promising preclinical (161, 162) and phase 1/2 clinical (163, 164) trials. Therapies with other targets of metabolism are currently undergoing clinical trials (165, 166). Perhaps more convincing evidence for metabolism as a mechanism of resistance will be found in the gut microbiota if microbial metabolites there modulate antitumor immune responses.

MICROBIOTA

The microbiome plays key roles in immune system development and education as well as disease pathogenesis (167, 168). It is now appreciated that the gut microbiome may affect responses to cancer immunotherapy. This insight was initially demonstrated in preclinical models of cancer where mice with distinct intestinal microbiota exhibited distinct responses to anti-PD therapy (169) or anti-CTLA-4 therapy (170). In human cancer patients, the gut microbiome was altered by anti-PD therapy and/or anti-CTLA-4 therapy (171, 172). Subsequently, three studies in 2018 demonstrated that human cancer patients can be stratified into responders and nonresponders to anti-PD therapy based on their intestinal microbiomes (173–175). Analysis of fecal microbiome samples from a cohort of 43 melanoma patients treated with anti-PD therapy revealed compositional differences between responders ($n = 30$) and nonresponders ($n = 13$) (173). Similar findings were obtained from a distinct cohort of 42 melanoma patients where different compositions of pretreatment fecal microbiome samples were associated with responders ($n = 16$) and nonresponders ($n = 26$) to anti-PD therapy (174). Interestingly, the specific bacterial species associated with response to anti-PD therapy in these melanoma cohorts were different, suggesting that there is no universal bacterial species associated with response, even in patients with the same cancer subtype. Routy et al. (175) demonstrated that gut microbiome composition is also associated with response to anti-PD therapy in NSCLC, renal cell carcinoma, and urothelial carcinoma. Furthermore, antibiotic exposure during immunotherapy negatively impacted response to anti-PD therapy (175). Intriguingly, these studies demonstrated that fecal microbiome transplants from responders resulted in greater efficacy of anti-PD therapy, with increased densities of $CD8^+$ T cells and reduced Tregs within the TME in germ-free mice. These studies and others have helped establish the importance of the gut microbiome as a potential source of a biomarker for primary resistance to anti-PD therapy across distinct cancers and the potential negative impact that antibiotics may have on patients undergoing anti-PD therapy (176–181). However, we do not understand the mechanism responsible for these associations, nor do we know whether microbiome alterations can contribute to secondary resistance to anti-PD therapy. Clinical trials specifically manipulating the gut microbiota in cancer patients are ongoing and will hopefully clarify the impact of the microbiota on immune responses to cancer (182, 183). In two recent phase 1 clinical trials, 25 melanoma patients who were resistant to anti-PD therapy underwent fecal microbiota transplantation prior to reintroduction of anti-PD therapy, and the results suggest that modulating the gut microbiome for immunotherapy is feasible and effective (184, 185) (**Figure 4*b***). Both studies used gut microbiota from patients that responded to anti-PD therapy. In one study with 10 patients, 3 responded with reduced tumor burden and more $CD8^+$ TILs. In the second study, 6 out of 15 melanoma patients responded to fecal microbiota transplantation and anti-PD therapy (185). Patients that responded had durable gut microbiome changes, increased $CD8^+$ T cell activation, and decreased IL-8-expressing myeloid cells (185). Use of probiotics to mitigate the effects of gut dysbiosis in patients who received antibiotics may be of benefit (178). Although the mechanisms responsible for the association of distinct bacterial taxa with response to anti-PD therapy are unknown, several preclinical models have suggested that microbial metabolites may be responsible for increasing IFN-γ production by T cells (186, 187). Other mechanisms have been proposed as well, such as the translocation of bacteria from the gut to the TME (188, 189), cross-reactivity between cancer antigens and microbial peptides (190), and direct presentation of bacterial peptides by cancer cells (191). These proposed mechanisms suggest that stimulation of antitumor immunity through the activation of innate immunity, lymphocyte licensing, or T cell stimulation is also important in overcoming resistance to anti-PD therapy. More work is needed in human cancer patients undergoing anti-PD therapy to determine whether the

microbiota plays a key role in resistance to therapy, and if so, how to target this mechanism of resistance.

EPIGENETICS AND ANTI-PD THERAPY RESISTANCE

Just as metabolism is a fundamental cellular process of critical importance to cancer cells and immune cells, so is modification of gene expression, or epigenetics. Control of gene expression occurs through epigenetic modifiers, including histone deacetylases, histone methyltransferases, DNA methyltransferases, and others. Epigenetic modifications drive T cell differentiation and function (192), thereby contributing to the antitumor immune response. Therefore, it is not surprising that modifications of epigenetics have been implicated in cancer immunotherapy (**Figure 4*c***). Expression of selective epigenetic modifiers is associated with cancer prognosis (193, 194), and epigenetic modifications can boost immunotherapy in mice (193–198). Moreover, dysfunctional T cells may be resistant to anti-PD therapy due to epigenetic stability (199). Approaches to modify the epigenome to boost responses to immunotherapy are currently undergoing clinical trials (200–203) based on preclinical studies (193–198). These include inhibitors of histone deacetylases, histone methyltransferases, and DNA methyltransferases. However, it is unknown whether targeting such a fundamental cellular process in combination with anti-PD therapy would be of benefit to cancer patients. For example, blockade of histone methyltransferase Ezh2 can either increase T cell responses (193, 194) or decrease T cell responses against cancers (204), likely due to differential effects on T cell subsets. In addition to the pleiotropic effects of Ezh2 on T cells, inhibition of Ezh2 has been shown to impair the function of DCs (205). There is currently not enough clinical evidence to support global epigenetic modifications as a mechanism of resistance to anti-PD therapy. Rather, selective epigenetic modifications may be contributing to mechanisms of immune resistance to anti-PD therapy. For example, antigen loss through promotor hypermethylation of neoantigens has been demonstrated in NSCLC patients (83). Additionally, loss of proteins involved in antigen presentation (β_2m) and interferon signaling and increase in expression of inhibitory immune receptors that drive anti-PD therapy resistance are likely regulated epigenetically. Finally, epigenetic modifications are responsible for maintaining T cells in a dysfunctional or exhausted state, limiting the response to anti-PD therapy (199).

OVERCOMING ANTI-PD THERAPY RESISTANCE

Overcoming resistance to anti-PD therapy is currently one of the major challenges in cancer immunotherapy and oncology (28). However, efforts should be guided by an understanding of the mechanisms underpinning resistance—a science-driven approach rather than random combination with available drugs and therapies. While our effort to overcome resistance to anti-PD therapy is ongoing and our understanding is far from perfect, several basic principles are emerging and are useful to guide treatment of cancer.

It is clear that anti-PD therapy mainly targets the TME. More work is needed to better define the dynamic and nuanced changes occurring within the TME. Therefore, obtaining tissue is critical for investigating mechanisms of resistance. Only longitudinal biopsies prior to treatment and during treatment will help distinguish the immune context of the TME in responders from that in nonresponders. New tools will be needed to interrogate the evolving immune response within the TME. Recent advances in single-cell technologies have helped identify key immune cells associated with response to anti-PD therapy (206–208). But most studies focus on only a few populations of cells isolated from the tumor and placed in suspension, ignoring the complex architecture of the TME. Next-generation histology allows for single-cell spatial proteomics,

including imaging mass cytometry (209), multiplexed ion beam imaging (210), and codetection by indexing (CODEX) (211), which provide unprecedented spatial resolution of the heterogeneity within the TME (212–214). Spatial resolution of gene expression through histology-based transcriptomic platforms such as digital spatial profiling (215) and spatial transcriptomics (216) has also illuminated new insights into the complex tumor-immune interface of cancers (217–219). These new tools may help identify new biomarkers associated with response to anti-PD therapy and mechanisms of resistance. Furthermore, visualizing the TME with single-cell spatial resolution may allow us to detect multiple mechanisms of resistance that operate simultaneously within the same tumor but are temporally and spatially segregated within microregions.

Predictive and Prognostic Biomarkers

Since the initial discovery of immune biomarkers associated with cancer prognosis, including T cell infiltration (220) and B7-H1 expression (20, 22, 34, 37), multiple signatures have been identified (221–223). With advances in single-cell technologies and computational biology, new biomarkers have emerged that help stratify patients based on anti-PD therapy responses, providing important prognostic insight (58, 224–227). Although tumor mutational burden was initially identified as a prognostic biomarker and predicted response to anti-PD therapy, subsequent studies have called into question the utility of tumor mutational burden as a biomarker of anti-PD therapy response (228). TME heterogeneity across cancer types and within the same cancer underlies the challenge in identifying a biomarker that has clinical utility for the prediction of anti-PD therapy responses. Given the complexity of immune evasion mechanisms, multiple biomarkers need to be identified to select the most appropriate immunotherapy to be used independently or in combination with anti-PD therapy.

Combination Therapy

One key approach to overcoming resistance to anti-PD therapy is combination with other drug targets to boost antitumor responses. Anti-CTLA-4 therapy is the most successful combination therapy. It is the standard of care for metastatic melanoma (229) and has shown benefit in other cancers (230), albeit with greater toxicities. Dual blockade of CTLA-4 and PD-1 increased overall five-year survival of melanoma patients (231). One of the major drawbacks of combination therapy with anti-CTLA-4 is severe systemic toxicity, which may help explain why the number of clinical trials using this combination is declining while combination trials overall are increasing (27). Hundreds of unique drug combinations with anti-PD therapy are being tested in thousands of clinical trials globally. An analysis of the anti-PD therapy clinical trial landscape for 2020 showed that among the 3,674 active clinical trials with anti-PD therapy, 2,949 (80%) were combination therapy trials (27). Anti-PD therapy was combined with 253 distinct drug-target groups, including immuno-oncology therapies (e.g., CTLA-4, oncolytic viruses, cancer vaccines, other immune inhibitory molecules), targeted therapies (e.g., VEGF), chemotherapy, and radiation (27). The accelerated growth of anti-PD therapy clinical trials, especially combination trials, is unlikely to falter anytime soon, as it has continued despite the COVID-19 (coronavirus disease 2019) global pandemic (232). Some combination treatment strategies directly inject immunostimulatory molecules into tumors (233, 234). We predict that, unfortunately, many, if not most, of these combination clinical trials will fail. This is because rational targeting of the local immune dysfunction within the TME is necessary to overcome immune evasion mechanisms and resistance to anti-PD therapy and optimize antitumor immune responses and enhance clinical outcomes. The success of anti-PD therapy above all other immunotherapies has taught us as

much. The approach of targeting the local dysfunctional immune response within the TME is referred to as normalization cancer immunotherapy (24, 25).

Normalization Cancer Immunotherapy

As discussed above, anti-PD therapy is the archetypal normalization cancer immunotherapy. The multiple immune evasion mechanisms used by tumors suggest that other key regulators are operational in the TME, and there is a great need to identify and target these mechanisms. There are three guiding principles to develop normalization cancer immunotherapies (24, 25). First, identify a local tumor immune escape mechanism occurring within the TME that is analogous to adaptive immune resistance. Second, selectively targeting immune dysfunction in the TME will limit systemic toxicity. Finally, successful targeting of the local immune evasion mechanism should reprogram a dysfunctional immune response to a normal antitumor immune response, eliminate cancer cells, and promote tissue homeostasis. Although anti-PD therapy fits these principles of normalization cancer immunotherapy better than other immunotherapies, it does so imperfectly. Patients treated with anti-PD therapy may experience systemic toxicity and incomplete eradication of cancers cells. Nevertheless, the principles of normalization cancer immunotherapy should serve as an aspirational framework for future therapies and rational clinical trial design.

CONCLUSIONS

Despite known inhibition of downstream TCR signaling pathways by PD-1, the precise mechanisms of effective anti-PD therapy remain largely unknown. Single-cell analyses of the TME response to anti-PD therapy have identified several potential key mechanisms involved in cancer eradication. As the critical T cell subsets and dendritic cell or macrophage subsets that most directly respond to anti-PD therapy are identified, it remains unclear how tissue homeostasis is restored or normalized. In many ways, we understand more about how anti-PD therapy fails to eradicate cancer than how it results in long-term cancer remission. In this review, we have focused on the recently identified mechanisms of resistance to anti-PD therapy.

The reader may have noticed that many mechanisms of resistance to anti-PD therapy are interconnected and dynamic and thereby difficult to untangle. We have attempted to classify mechanisms of resistance to anti-PD therapy into discrete categories such as immune, microbiota, metabolism, and epigenetics for conceptualization, but resistance often involves many of these categories with bewildering complexity. Additional mechanisms of resistance not discussed in this review will certainly be uncovered in future studies, and new challenges to immunotherapy will need to be addressed. Several questions remain: How many mechanisms of anti-PD therapy are operating within a single patient or even a single metastatic lesion within the same patient? What is the temporal therapeutic window when strategies to overcome specific resistance mechanisms may be implemented? Can we identify mechanisms of resistance prior to initiating therapy? Unique mechanisms of resistance to immunotherapies beyond anti-PD therapy will also need to be interrogated to enable strategies to prevent or treat them and improve clinical outcomes. Nevertheless, the transformative success of targeting the TME with anti-PD therapy provides continued hope and optimism that medicine, academia, and industry will cooperate to overcome these challenges.

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AUTHOR CONTRIBUTIONS

M.D.V., T.Z., and L.C. contributed to conceptualization. L.C. supervised. M.D.V. and T.Z. contributed to developing the figures. M.D.V. and T.Z. wrote the original draft. M.D.V., T.Z., and L.C. participated in writing, reviewing, and editing the manuscript.

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