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Mechanisms of Resistance to PD-1 and PD-L1 blockade

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Abstract

Cancer immunotherapy utilizing blockade of the PD-1/PD-L1 checkpoint has revolutionized the treatment of a wide variety of malignancies, leading to durable therapeutic responses not typically seen with traditional cytotoxic anti-cancer agents. However, these therapies are ineffective in a significant percentage of patients, and some initial responders eventually develop resistance to these therapies with relapsed disease. The mechanisms leading to both primary and acquired resistance to PD-1/PD-L1 inhibition are varied, and can be both multifactorial and overlapping in an individual patient. As the mechanisms of resistance to PD-1/PD-L1 blockade continue to be further characterized, new strategies are being developed to prevent or reverse resistance to therapy, leading to improved patient outcomes.

Keywords

Immunotherapy; PD-1; PD-L1; resistance

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INTRODUCTION

Immunotherapy represents a paradigm shift in the treatment of cancer, particularly the inhibition of various checkpoints that control host T cell activity. Programmed cell death 1 (PD-1) interacting with its corresponding ligand PD- L1 has emerged as a common means by which cancer cells evade the host immune response (1). PD-1 is expressed on activated $CD8^+$ T cells (as well as B cells and natural killer cells) in the setting of chronic antigen exposure. The interaction between PD-1 and PD-L1 or PD-L2 on host tissues leads to the inhibition of T cell receptor (TCR) signaling and CD28 co-stimulation (2, 3), limits T cell interactions with target cells, and ultimately leads to their inactivation and loss of proliferative capacity (4–7). PD-L1 expression is induced by localized inflammatory stimuli, such as interferons (IFNs) released by the infiltrating T cells (5). The PD-L1 induction process in cancer has been termed “adaptive immune resistance” (1), and represents a mechanism by which cancer cells protect themselves from T cell mediated destruction.

This new approach to treating cancer has led to the clinical development of therapeutic monoclonal antibodies blocking PD-1 or PD-L1. Pembrolizumab (Keytruda®) and nivolumab (Opdivo®) are two such agents targeting PD-1, while atezolizumab (Tecentriq®), avelumab (Bavencio®), and durvalumab (Imfinzi®) block PD-L1 instead (8). Collectively, these agents are approved for the treatment of a wide variety of malignancies, including metastatic melanoma, non-small-cell lung cancer (NSCLC), head and neck squamous cell cancer, Hodgkin’s lymphoma, renal cell carcinoma (RCC), urothelial carcinoma, Merkel cell carcinoma, gastric carcinoma, and hepatocellular carcinomas (8–22). These agents inhibit the negative regulatory effects of PD-L1 on patient T cells via PD-1, resulting in the enhancement of a pre-existent antitumor immune activity. This unleashes a focused T cell response against a patient’s tumor, as it increases proliferation of tumor infiltration lymphocytes (TILs), and leads to a more clonal TCR repertoire within the T cell population directed against the tumor (23). These effects ultimately provide patients with significant and durable immune responses against their malignancy, with complete responses lasting for years in some cases.

Although PD-1/PD-L1 checkpoint blockade can result in dramatic therapeutic responses, this therapy is only effective in a subset of patients, and many patients are only partial responders to therapy (9, 12, 24). Patients who do not respond to initial therapy with PD-1/PD-L1 blockade are referred to as having “primary resistance” to therapy (25). Furthermore, there is a growing subset of patients who, despite showing a robust initial response to therapy, go on to develop progressive disease. This phenomenon, in which disease is either refractory to resumption of therapy, or develops despite continuation of therapy, is known as “acquired resistance” to PD-1/PD-L1 blockade immunotherapy (25, 26). Both phenomena are highly complex, as the mechanisms for both types of resistance can be overlapping and/or multifactorial. Furthermore, each patient’s individual environmental and genetic factors, as well as prior treatments, can create an evolving therapeutic landscape unique to a given patient. As the mechanisms responsible for both primary and acquired resistance to PD-1/PD-L1 blockade are further elucidated, improved treatments with superior therapeutic efficacy can be developed. This review will explore the

mechanisms responsible for primary and acquired resistance to immunotherapy via PD-1/PD-L1 blockade, as well as techniques being studied to overcome such resistance.

PRIMARY RESISTANCE TO PD-1/PD-L1 BLOCKADE

Patients treated with PD-1/PD-L1 blockade who never demonstrate a clinical response or stabilized disease are referred to as having "primary resistance" to therapy. Early data from clinical trials demonstrated that the presence of pre-existing $CD8^+$ T cells within the tumor and its periphery, along with co-localized PD-1 and PD-L1 expression on the T cells and tumor cells, respectively, predicted therapeutic response to anti-PD-1 therapy in patients with malignant melanoma (23). Therefore, many studies have explored primary resistance as a phenomenon in which a patient's $CD8^+$ T cells are either unable to recognize and localize to the tumor, or are rendered ineffective despite seemingly adequate localization. The latter mechanism can occur due to other cell types that exert local immunosuppressive effects within the tumor microenvironment, or by inherent resistance of tumor cells to the effects of the T cells themselves.

Inadequate T cell infiltration due to lack of tumor immunogenicity

The inability of host $CD8^+$ T cells to localize to a tumor can be most simply attributed to an absence of sufficiently immunogenic tumor antigens for T cell recognition (27). This may be the case in tumors that are either not significantly dedifferentiated from their tissue of origin, or possess insufficient mutational burden to express tumor antigens which are able to produce a focused $CD8^+$ T cell response. The resulting absence of T cells that can differentially recognize unique tumor antigens renders such tumors non-responsive to PD-1/PD-L1 blockade therapy. Indeed, tumors with high mutational burden and increased tumor neoantigen expression, such as melanoma, head and neck, NSCLC, bladder, and microsatellite unstable cancers are generally more responsive to anti-PD-1/PD-L1 therapy (12, 28–30). RCC, which has a relatively low mutational burden, appears to be a notable exception to this trend (31), demonstrating the importance of other factors in its responsiveness. However, tumors with few somatic mutations, such as pancreatic and prostate cancers, are generally more resistant to PD-1/PD-L1 blockade (28, 32). One approach to overcome this obstacle is to supply with the patient with T cells targeting antigens that might not inherently display strong immunogenicity. Adoptive cell therapy (ACT) involves the generation of large quantities of $CD8^+$ T cells directed at specific tumor antigens, such as MART-1 in melanoma and NY-ESO-1 in sarcoma (33–35). T cells specific for the tumor antigen can be generated by *ex vivo* expansion of endogenous, low-frequency $CD8^+$ T cells specific for a characterized tumor antigen, which are then re-infused into the patient (36, 37). Alternatively, such cells can be generated by *ex vivo* genetic modification of peripheral mononuclear blood cells, which are transduced with a TCR directed against a given tumor antigen, expanded, and re-infused into the patient (34). Tumor vaccines are also an effective means of stimulating patients' dendritic cells to generate T cell activity against tumor antigens (38), including with the use of mutational neoantigens as vaccines (39, 40). Combining ACT or dendritic cell vaccines with PD-1 inhibition can be effective in generating a therapeutic response in these cases by providing a focused T cell response against the tumor (41–44). More novel ways of inducing T cell infiltration within tumors to

sensitize them to PD-1 inhibition have also been studied. Ribas et al recently reported the novel combination of intralesional injection of a modified human herpes simplex virus with systemic anti-PD-1 therapy for the treatment of melanoma in a phase 1b clinical trial (45). This lead to a 62% objective response rate, as well as 33% complete response rate in patients, and was accompanied by enhanced T cell infiltration in virus-injected lesions. Additionally, responses to this combination appeared to be independent of baseline $CD8^+$ T cell infiltration. As new methods of stimulating T cell infiltration within tumors are characterized, their combination with anti-PD-1/PD-L1 therapies may continue to lead to improved response rates.

Intracellular antigens require cell surface presentation via the major histocompatability complex (MHC) and a lack of antigen presentation can lead to tumor resistance to T cells. Various mechanisms to disrupt the machinery of antigen presentation have been described including mutations that interfere with the proteasome, transporters associated with antigen processing (TAP), and the structural components of the MHC itself (46). Several groups had demonstrated long ago that mutations resulting in loss of beta-2-microglobulin (β2M) could render melanoma tumors resistant to T cell infiltration, due to the role β2M plays in MHC transport and stable expression on the cell surface (47–49). The resultant inability to present antigens via MHC class I renders the tumor cells effectively undetectable by $CD8^+$ T cells. Antigen processing and surface presentation via the MHC can be disrupted by epigenetic changes as well, such as hypermethylation and histone acetylation (50, 51). Therefore, agents reversing these processes may augment T-cell dependent immunotherapy with PD-1 (52). Demethylating agents and histone deacetylation agents alike have been shown to increase the MHC presentation of tumor-specific antigens in models of lymphoma and melanoma, leading to increased infiltration by $CD8^+$ T cells and subsequent tumor responses (53, 54). As the ability to classify epigenetic modulation status of individual tumors becomes better refined and more specific epigenetic drugs are being developed, combinatorial strategies involving epigenetic modification therapy with PD-1/PD-L1 blockade may be effective in overcoming these cases of primary resistance.

T cell exclusion

Primary mutagenesis events within a tumor can also lead to a phenomenon known as T cell exclusion, in which T cell tracking to the tumor microenvironment is inhibited without impacting antigen expression or presentation. One mutation reported to contribute to T cell exclusion is activation of β-catenin/Wnt signaling. In a mouse model of melanoma, Spranger et al demonstrated an inverse correlation between β-catenin/Wnt activation in tumors and their degree of $CD8^+$ T cell infiltration (55). Increased β-catenin/Wnt activity was also associated with decreased $CD103^+$ dendritic cell infiltration due to decreased CCL4 expression, a chemokine responsible for their attraction. These melanoma tumors with β-catenin/Wnt activation responded poorly to PD-1 blockade therapy, whereas tumors without β-catenin/Wnt mutations responded well to treatment with PD-1 blockade. β-catenin/Wnt expression has also been correlated with T cell exclusion in urothelial bladder cancer, another tumor type which can be treated by PD-1/PD-L1 inhibition (56). Further study regarding this mechanism of T cell exclusion can lead to better classification of the likelihood of a tumor's response to checkpoint blockade. Several drugs inhibiting

constitutive β-catenin/Wnt pathway signaling are on clinical development, and can be combined with anti-PD-1/L1 therapy to overcome this mode of primary resistance.

Other mutations associated with T cell exclusion and subsequent resistance to PD-1/PD-L1 blockade are those within the mitogen activated protein kinase (MAPK) signaling cascade. Constitutive oncogenic signaling through this pathway results in the production of the immunosuppressive cytokines vascular endothelial growth factor (VEGF) and interleukin 8 (IL-8), which inhibit T cell recruitment to the tumor, as well as their functionality (57, 58). Activating mutations within the MAPK cascade are common in melanoma, and inhibition of this cascade has been shown to both improve $CD8^+$ T cell infiltration within tumors, as well as sensitize them to PD-1 blockade therapy (59). These data have provided strong rationale for combination therapy of multi-kinase inhibition with PD-1 blockade in tumors with such mutations. Similarly, loss of the tumor suppressor phosphatase and tensin homolog (PTEN), which leads to activation of phosphatidylinositol 3-kinase (PI3K) signaling, has also been associated with increased VEGF production and reduced $CD8^+$ T cell infiltration of tumors and subsequent resistance to PD-1 blockade therapy. In clinical samples of melanoma with loss of functional PTEN, Peng et al showed a decrease in T cell infiltration proportional to the frequency of PTEN deletions and mutations. Furthermore, in a murine model, addition of a PI3K inhibitor to PD-1 blockade produced superior regression of PTEN-deficient tumors (60). These studies demonstrate the need for continued study of targetable mutations leading to primary resistance to PD-1/PD-L1 blockade, which can lead to clinical therapeutic regimens combining kinase inhibitor therapy with immune checkpoint blockade to improve response rates.

Tumor cell resistance to interferon

Tumors can display inherent primary resistance to PD-1/PD-L1 blockade despite effective CD8 T cell recognition. This can occur due to lack of interferon-gamma (IFNγ) responsiveness in a tumor, which can occur in the setting of both primary and acquired resistance to checkpoint inhibition therapy. IFNγ is produced by $CD8^+$ T cells which have recognized and engaged an appropriate tumor antigen, and represents a primary means of increasing MHC expression/antigen presentation, recruiting additional T cells to tumors, and inducing direct anti-proliferative effects and apoptosis in cancer cells (61). Furthermore, this IFNγ stimulation is a primary cause of the induction of PD-L1 on the tumor cells themselves, as previously discussed (62, 63). IFNγ binds to the interferon-gamma receptor (IFNGR), which leads to JAK1 and JAK2 activation and subsequent STAT1 and STAT3 recruitment and phosphorylation (64). This complex then translocates to the nucleus, where it activates interferon regulatory factor 1 (IRF1), the transcriptional activity of which ultimately gives rise to the anti-tumor effects of IFNγ, as well as PD-L1 expression (62, 63, 65). Primary mutations resulting in loss of function of JAK1 and JAK2 have been shown to impair the IFNGR signaling pathway, resulting in loss of the anti-tumor effects of IFNγ (66–68). This phenomenon also results in decreased or absent PD-L1 expression by the tumor cells. However, rather than augmenting the $CD8^+$ T cell response against the tumor by removing the inhibitory effect of PD-L1 on the T cells via PD-1, such cancer cells with JAK1/2 mutations display primary resistance to anti-PD-1 therapy by virtue of their overall resistance to the anti-tumor effects of IFNγ itself (68). Additionally, in a preclinical model

of CRISPR screens, mutations in the apelin receptor, which interacts with JAK1 to modulate IFNγ responses, have also been associated with primary resistance to immunotherapy with PD-1 blockade via IFNγ resistance (69). Furthermore, in another CRISPR screen, activating mutations in tyrosine-protein phosphatase non-receptor type 2 (Ptpn2), which negatively regulates JAK1 and STAT1 signaling, have also been associated with primary resistance to PD-1 blockade via resistance to IFNγ, and deletion of Ptpn2 via CRISPR-Cas9 genome editing was able to restore IFNγ sensitivity in a melanoma model (70). Further classification of tumors at the genetic level can provide evidence of functional IFNγ response elements, which would be a prerequisite for an effective response to PD-1/PD-L1 blockade therapy.

In addition to reflecting dynamic IFNγ responsiveness, PD-L1 expression can also be constitutively expressed in some cases. Although the prognostic implications of constitutive expression of PD-L1 are not always clear, there are some that are associated with poor response to therapeutic inhibition of the PD-1/PD-L1 checkpoint. In patients with NSCLC, patients with EGFR mutations and ALK rearrangements have poor response rates to PD-1/PD-L1 blockade. Patients with such mutations display high PD-L1 expression rates despite low or absent $CD8^+$ T cell infiltration, implying that the PD-L1 expression was constitutive rather than induced by local inflammatory stimuli; these tumors correspondingly demonstrated a lack of responsiveness to PD-1/PD-L1 blockade (71). Additionally, preclinical models of NSCLC harboring EGFR mutations and ALK rearrangements have demonstrated that these genetic aberrations are directly responsible for the constitutive PD-L1 expression seen in these tissues (72, 73). Although the mechanism for the apparent immune exclusion caused by these mutations remains unclear, they nevertheless underscore the danger in predicting a tumor as being responsive to PD-1/PD-L1 blockade therapy based solely on the expression of a single marker.

Local immunosuppressive factors within the tumor microenvironment

The wide variety of other cell types within the tumor microenvironment (TME) represents another diverse pool of potential modulators of immune activity against a tumor. Certain non-tumor cells within the TME can impair a response to PD-1/PD-L1 blockade despite seemingly adequate infiltration by $CD8^+$ T cells and interferon-responsive tumor cells. One of these is the myeloid-derived suppressor cell (MDSC), a heterogeneous population of immature myeloid cells recruited by tumors with the ability to regulate local immune function. MDSCs dampen T cell responses via multiple mechanisms, including depletion of local nutrients, production of reactive oxygen species, and nitrosylation of local chemokines (74–77). Several studies have demonstrated that depletion of intra-tumor MDSCs restores the effectiveness of PD-1 blockade (78, 79). Another immunosuppressive cell type within the TME is the regulatory T (Treg) cell. Treg cells are a subtype of $CD4^+$ T cells that suppress the activity and proliferation of local effector CD8 T cells; when present within the TME, this results in a poor immunologic response against the tumor (80, 81). Strategies which deplete or impair Tregs have led to improved responsiveness to PD-1/PD-L1 checkpoint blockade (82, 83). Further studies characterizing intra-tumor Tregs and MDSCs and their inhibition will aid in overcoming this mechanism of primary resistance to checkpoint blockade.

Tumor cells themselves and their surrounding stroma can modulate immunosuppressive states within the TME, resulting in tumors with primary resistance to PD-1/PD-L1 blockade therapy. Enrichment of a collection of genes termed innate anti-PD-1 resistance (IRPES) has been characterized in patients with melanoma who were non-responsive to checkpoint blockade (84). These include locally immunosuppressive genes (VEGF, IL-10), as well as genes responsible for mesenchymal transition, monocyte/macrophage/MDSC chemotaxis, wound healing, and angiogenesis. IPRES signatures, interestingly, represent a transcriptomic program that exists across multiple cancer subtypes, including melanoma, kidney clear cell carcinoma, colon adenocarcinoma, lung adenocarcinoma, and pancreatic adenocarcinoma (84). Correlation of such patterns across other malignancies may provide a distinct means of predicting a tumor's response to immunotherapy with PD-1/PD-L1 blockade.

ACQUIRED RESISTANCE TO PD-1/PD-L1 BLOCKADE

Immunotherapy with PD-1/PD-L1 blockade has been characterized by a subset of patients who exhibit long-lasting responses to treatment. However, over time, cohorts of patients have emerged who either eventually progress while on therapy despite an initially robust response, or who are unresponsive to re-initiation of checkpoint blockade. The mechanisms behind acquired resistance to therapy are diverse, and can occur through similar mechanisms responsible for primary resistance. Broadly, the pathogenesis of acquired resistance to anti-PD-1/PD-L1 therapy can include eventual loss of T cell function (due to epigenetic dysfunction or the acquisition of other immunosuppressive signals), disruption of antigen presentation (leading to decreased T cell recognition of the tumor), and development of resistance to the effects of interferon generated by the T cells.

Loss of T cell function

PD-1 expression occurs on $CD8^+$ T cells in the setting of persistent antigen stimulation, and its stimulation leads to impaired effector functionality within these cells. PD-1 blockade can re-invigorate these hypofunctional "exhausted" $CD8^+$ T cells (T_{EX}), restoring their anti-tumor effector functionality (85). However, recent data have elucidated the degree to which this phenomenon is sustainable *in vivo*, which has significant implications for relapse to PD-1 blockade therapy. Tumor-infiltrating T_{EX} cells can be characterized by increasing dysfunction driven by progression through discrete epigenetic states. While initially plastic and able to be modified to restore an effector functionality, they eventually reach a state of fixed epigenetic dysfunction, in which their chromatin is rendered inaccessible and resistant to further remodeling and reinvigoration (86). The overall ratio of reinvigorated T_{EX} to pretreatment tumor burden has also been correlated with degree of clinical response to PD-1 blockade (87). If the tumor burden remains high and reinvigorated T_{EX} fail to clear the tumor, they eventually become re-exhausted, acquiring a fixed epigenetic state that renders them resistant to reinvigoration via PD-1 blockade (88). Additionally, PD-1 blockade in T_{EX} activates transcriptional changes associated with effector T cells (T_{EFF}), but not with memory T cells (T_{MEM}) (88). T_{MEM} have greater proliferative capacity and longevity than T_{EFF}, and are associated with greater long-term potentiation of immune responses, including those directed against tumors (89–91); a lack of T_{MEM} cells combined with re-exhausted T_{EX} leads to tumor progression after an initial response. This phenomenon underscores the

need for development of epigenomic modification strategies which can perpetuate reinvigoration of T cells in response to PD-1/PD-L1 blockade, resulting in a more sustainable anti-tumor activity.

In addition to PD-1, there are other immunosuppressive signaling receptors that can impair anti-tumor T cell functionality. These include cytotoxic T-lymphocyte antigen 4 (CTLA-4), T-cell immunoglobulin and mucin domain-containing molecule-3 (TIM3), lymphocyte activation gene-3 (LAG-3), and V-domain Ig suppressor of T cell activation (VISTA), among others (92, 93). While their expression can occur at any time, TIM3 has been shown to be up-regulated in patients with NSCLC who developed acquired resistance to anti-PD-1 therapy (94, 95). The resistance of these tumors to anti-PD-1 therapy was able to be overcome in a murine model with the addition of a TIM-3 blocking antibody. Up-regulation of VISTA on TILs over time has also been demonstrated in cases of acquired resistance to PD-1 blockade (96). While the expression patterns of these markers and their correlation with response to immunotherapy are still being characterized, blocking multiple immune inhibitory checkpoints is a logical extension of these data. Several trials are currently underway testing the effectiveness of combining PD-1/PD-L1 blockade with inhibitors of other co-existing immune regulatory checkpoints as a means to overcome resistance to therapy (97, 98). Indeed, the clinical combination of the anti-CTLA-4 antibody ipilimumab with nivolumab (an anti-PD-1 antibody) is FDA-approved for the treatment of metastatic melanoma, and has demonstrated superior outcomes compared to nivolumab alone (99, 100). As the expression patterns of other immunosuppressive factors are further characterized, future immunotherapy regimens can be designed combining multiple levels of checkpoint blockade to improve clinical outcomes and overcome resistance to therapy.

Disruption of antigen presentation

As previously discussed, antigen/neoantigen expression and presentation are vital to T cell recognition of a tumor and engagement of the TCR. While primary mutagenesis events can interfere with this process in a variety of ways and contribute to resistance, this can also occur following initiation of immunotherapy, resulting in acquired resistance to PD-1/PD-L1 blockade. Inflammatory stress has been shown to induce melanoma cell dedifferentiation along the neural crest lineage, resulting in increased expression of the nerve growth factor receptor (NGFR) and decreased expression of melanosomal antigens such as MART-1 (101–103). This is also true of mutational neoantigens, which are especially important in T cell recognition of tumors in the setting of checkpoint blockade (28, 104). This has recently been demonstrated in NSCLC, where patients who developed acquired resistance to PD-1/PD-L1 blockade were found to have lost multiple mutation-associated neoantigens while on treatment (105). These neoantigens, which had been present in the tumors prior to initiation of checkpoint blockade, were found to have superior MHC binding affinity than the remaining neoantigens present at disease progression. Furthermore, the lost neoantigens were found to induce clonal T cell expansion *in vitro*, demonstrating their ability to elicit a T cell response. These findings demonstrate the need for better understanding of how the neoantigen landscape changes in patients who develop acquired resistance to PD-1/PD-L1 blockade. Classification of putative neoantigens in real time could provide early evidence of patients more likely to develop resistance to therapy.

Antigen/neoantigen presentation can also be disrupted via mutations in the antigen presenting machinery. While this can occur at baseline in the setting of primary resistance to PD-1/PD-L1 blockade, it has been shown to occur to patients while on treatment as well. Loss of β2M, which is required for surface expression of class I MHC, has been demonstrated to occur following initiation of adoptive cell therapy or IL-2 treatment, leading to loss of T cell recognition of the tumor (48). This same phenomenon has been demonstrated in clinical examples of acquired resistance to PD-1 blockade in melanoma (106), in which a homozygous truncating mutation in β2M led to lack of surface expression of MHC class I. This mutation was not present in the baseline tumor, and ultimately led to acquired resistance to therapy in the patient. This demonstrates the ability of immunotherapy with PD-1/PD-L1 blockade to induce mutations that allow a tumor cell to escape detection by the T cells. Such tumor cells are then clonally expanded via selective pressure, resulting in disease relapse in patients who had initially responded to therapy.

Evolution of interferon resistance

Interferon responsiveness of a tumor is vital to the success of any T cell-based immunotherapy, including PD-1/PD-L1 blockade. While mutations within interferon signaling elements have been described in the setting of primary resistance to therapy, these mutations can also develop following initiation of therapy. Zaretsky et al demonstrated that patients with melanoma who developed resistance to anti-PD-1 therapy had acquired loss-of-function mutations in JAK1 and JAK2 (106), similar to the reported mechanisms responsible for primary resistance to therapy involving mutations in this signaling cascade (68). Although the tumor cells could still be recognized and engaged by T cells, their JAK1/2 mutations rendered them resistant to the effects of interferon stimulation. Tumor cells from these patients displayed absent activity in the IFNγ signaling pathway, insensitivity to the anti-proliferative effects of IFNγ, and lack of IFNγ-induced surface expression of both PD-L1 and MHC class I. As with disruption of antigen presentation machinery, this phenomenon underscores the ability of PD-1/PD-L1 blockade to clonally select for cells that can develop new mutations capable of withstanding inflammatory stresses brought on by the anti-tumor T cells.

CONCLUSIONS

Although immunotherapy with PD-1/PD-L1 blockade has shown great promise for the treatment of a variety of advanced cancers, significant durable responses only occur in a minority of patients, and patients who initially respond can ultimately relapse despite continued treatment. The reasons for response or resistance to therapy are multifaceted, highly individualized, and can evolve over time during treatment. There remains a critical need for the development of comprehensive monitoring of patients being treated with these agents. As the mechanisms responsible for resistance continue to be characterized, therapies can be personalized to optimize a patient's chance of responding, and real-time changes to therapeutic regimens can be made to overcome relapse. These highly individualized approaches in response to therapeutic monitoring will ultimately allow many more patients to benefit from these agents.

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