

## Molecular Pathways: Next-Generation Immunotherapy— Inhibiting Programmed Death-Ligand 1 and Programmed Death-1

Daniel S. Chen<sup>1,2</sup>, Bryan A. Irving<sup>2</sup>, and F. Stephen Hodi<sup>3</sup>

### Abstract

The aim of T-cell-based immune therapy for cancer has been to generate durable clinical benefit for patients. Following a generation of therapies that largely showed minimal activity, substantial toxicity, and no biomarkers to identify which patients benefit from treatment, early studies are showing signs that programmed death-ligand 1 (PD-L1) and programmed death-1 (PD-1) inhibitors are highly active. Preclinical and early data from clinical studies suggest that targeting this pathway can induce durable clinical responses in patients in a variety of tumor types, including lung and colon cancer. Furthermore, correlations with tumor PD-L1 expression may enable selection of patients most likely to benefit from treatment. The emerging data not only offer the hope of better cancer therapy but also provide evidence that changes our understanding of how the host immune system interacts with human cancer. *Clin Cancer Res*; 18(24): 6580–7. ©2012 AACR.

### Background

In the era of targeted therapy for cancer, drug development has focused primarily on identifying and targeting overexpressed or mutationally activated proteins that malignant cells require for continued survival and growth. While this approach has led to the development of numerous successful therapies, cancer is generally composed of a multitude of mutations, which render it highly adaptable (1). In the setting of metastatic disease, clones of cancer cells that have reduced or no dependence on the therapeutic target will most often emerge or evolve (2). The goal for cancer immunotherapy has long been to harness and redirect the host immune response. Once activated, the highly adaptable host immune response (3, 4) can recognize and kill cancer cells and potentially match the cancer's capability to evolve, leading to the hope of durable responses.

### Multiple Immunologic Nodes

The generation of an effective antitumor immune response is a complex multistep process, the understanding of which provides rational immunotherapeutic strategies (Fig. 1; refs. 4–6).

The T cells of the immune system must first be able to recognize cancer cells as abnormal, generate a population of

cytotoxic T lymphocytes (CTL) that can traffic to and infiltrate tumors wherever they reside, and specifically bind to and then kill cancer cells. Each step of this process must be met to achieve clinical benefit. Emerging clinical data highlight the importance of one inhibitory ligand and receptor pair, programmed death-ligand 1 (PD-L1; B7-H1 and CD274) and programmed death receptor-1 (PD-1; CD279), in inhibiting the last step of this process and preventing killing of cancer cells by CTLs.

### PD-L1/PD-1 Pathway

Tumors expressing PD-L1 can render CTLs inactivated or nonfunctional through engagement of the inhibitory receptor PD-1. PD-1 is expressed on the surface of T cells upon activation (7, 8), and binding of PD-L1 to PD-1 delivers an inhibitory signal, reducing cytokine production and proliferation of T cells (9, 10). In this fashion, PD-L1 expression by cells can mediate protection against CTL killing and represents a regulatory mechanism that potentially develops to dampen chronic immune responses during viral infections (11). Cancer, as a chronic and often inflammation-provoking disease, likely evolves to use this immune protective pathway during disease progression through upregulation of PD-L1 expression as a means to evade the host immune response.

PD-L1, the predominant ligand for PD-1, is expressed broadly in multiple tissues including T and B cells, dendritic cells, and macrophages. PD-L1 is frequently found to be highly expressed in many human cancer types (Table 2), being upregulated in tumors by activation of key oncogenic pathways [e.g., phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) or most strongly by IFN- $\gamma$  produced in the tumor microenvironment by an active antitumor T-cell response; refs. 3, 4, 12]. PD-L2

**Authors' Affiliations:** <sup>1</sup>Stanford Medical Oncology, Stanford; <sup>2</sup>Genentech, South San Francisco, California; and <sup>3</sup>Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

**Corresponding Author:** F. Stephen Hodi, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Phone: 617-632-4715; Fax: 617-582-7992; E-mail: stephen\_hodi@dfci.harvard.edu

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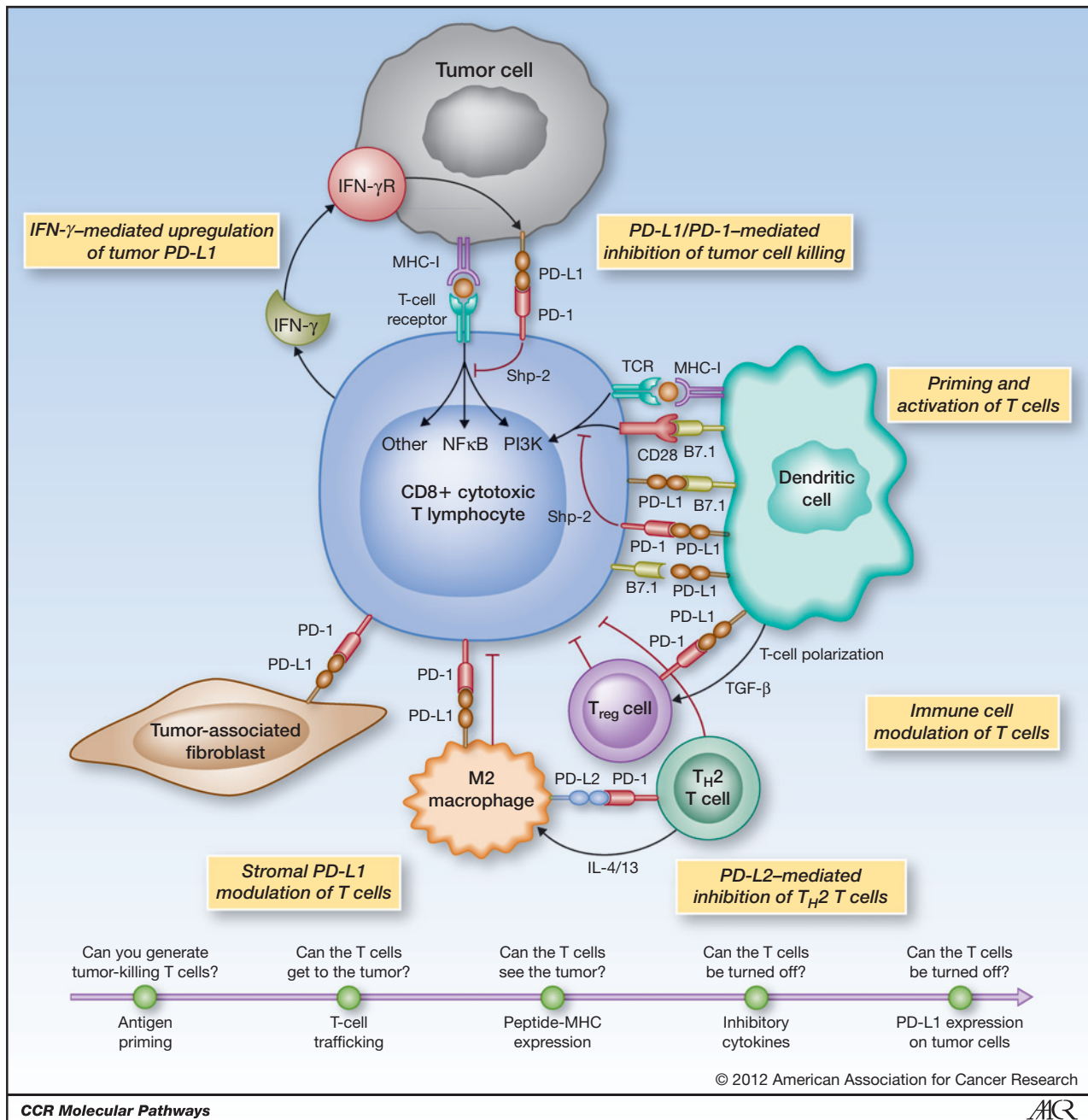


Figure 1. Tumor immunology and the PD-L1/PD-1 pathway.

(B7-DC and CD273), which similarly acts to dampen T-cell responses via engagement of PD-1 (13, 14), shows much more restricted expression, being primarily expressed on dendritic cells (Fig. 1), macrophages, and bone marrow-derived mast cells. Differences in expression patterns suggest distinct functions in immune regulation across distinct cell types. The restricted expression of PD-L2, largely to antigen-presenting cells, is consistent with a role in regulating T-cell priming or polarization, whereas broad distribution of PD-L1 suggests a more general role in protecting peripheral tissues from exces-

sive inflammation. Similar to its more restricted tissue distribution, PD-L2 expression across tumor types is far less prevalent than PD-L1.

In addition to PD-1, PD-L1 may also mediate an immunosuppressive function through its interaction with other proteins, including B7.1, potentially blocking its ability to activate T cells through binding to CD28, further dampening the generation of an immune response (15). Recent investigations using antibodies that selectively block the PD-L1/B7-1 interaction or that target PD-L1 in the absence of PD-1 show enhanced T-cell responses (16–18).

**Table 1.** Fc domain considerations for developing mAbs targeting the PD-1/PD-L1 pathway

	IgG1	IgG4	IgG1 - effectorless
ADCC	Optimal	Reduced	None
Binds complement	Yes	No	No
Antitumor activity	Favors direct	Little direct	Mediated solely through immune modulation
PD-1 expression (PD-1 expressed on activated T cells)	May kill PD-1-expressing TILs	May kill PD-1-expressing TILs	Does not kill PD-1-expressing TILs
PD-L1 expression (PD-L1 expressed on activated T cells, Tregs, tumor cells)	May kill PD-L1-expressing TILs	May kill PD-L1-expressing TILs	Does not kill PD-L1-expressing TILs

Abbreviations: TIL, tumor-infiltrating lymphocytes; Treg, regulatory T cells.

### Clinical-Translational Advances

The clinical significance of the PD-L1/PD-1 axis in inhibiting effective antitumor immunity is highlighted by trials testing fully human blocking antibodies. This clinical experience has included hundreds of treated patients and has shown clear efficacy signals in early studies. Across clinical trials that have examined antibody blockade of PD-L1 or PD-1, preliminary data support the ability to generate durable responses or disease control, with many responses ongoing beyond 2 years. The observed efficacy has been most clearly defined by monotherapy clinical objective responses, which can occur in approximately one-third to one-fifth of patients with metastatic melanoma, renal cell carcinoma (RCC), and non-small cell lung cancer (19–22). Additional responses have also been reported in metastatic colorectal cancer and prostate cancer. While these responses alone, observed primarily in a phase I patient population, are of particular interest, it is the initial durability of these responses being observed that may provide the greatest significance. The responders in the initial phase I study of a PD-1 inhibitor included a complete response (colorectal cancer) beyond 4 years, a partial response (RCC), which evolved into a complete response beyond 3 years, and a partial response (melanoma) that recurred at approximately 3 years and was successfully retreated (6, 23–25). Furthermore, analysis of available tumor tissue suggests that these responses were observed specifically in patients whose tumors expressed PD-L1, leading to a hypothesis that patients most likely to benefit from treatment may be identified prospectively by a diagnostic test (19, 20).

The emerging clinical data support the notion that agents targeting the PD-L1/PD-1 pathway can induce antitumor activity early during treatment and across multiple cancer indications. This suggests that for many human cancers, an underlying host immune response against an existing tumor does exist but is being restrained primarily by PD-L1 tumor expression. Although host immune surveillance may prevent tumor outgrowth during the earliest stages of tumor growth, locally invasive or metastatic tumors must evade host immunity.

### Strategies to Target the PD-L1/PD-1 Axis

#### Targeting PD-1 in cancer

Antibodies against PD-1 (or PD-L2-Fc fusion proteins) should inhibit binding of both PD-L1 and PD-L2 to PD-1 expressed on activated T cells, thus freeing tumor antigen-specific CTLs to mediate killing (4, 19, 26). This strategy may be particularly appropriate for tumors that express high levels of both PD-1 ligands. Another potential advantage of directly targeting PD-1 is the possibility that PD-1 can be more efficiently saturated on T cells in the blood before they migrate into tumors or other sites such as the central nervous system (CNS), which might otherwise require higher circulating concentrations of antibodies to derive optimal therapeutic benefit.

However, directly inhibiting PD-1 also raises the potential of increased inflammatory toxicity. Both PD-L1 and PD-L2 have been implicated in playing an important role in maintaining immune homeostasis, particularly in the lung (27). Serious and sometimes fatal lung-related autoimmune adverse events have been reported in studies of an antibody targeting PD-1 (28), and improved understanding of this toxicity will need to be further characterized in clinical studies.

#### Targeting PD-L1 in cancer

Antibodies that target PD-L1 act primarily through inhibiting the binding of PD-L1 to PD-1, freeing cancer antigen-specific CTLs to mediate killing of PD-L1-expressing cancer cells (refs. 4, 29; clinicaltrials.gov NCT01656642, NCT01633970, NCT01375842). This approach focuses on the pathway that many human cancers use to evade immune destruction: upregulation of PD-L1 on tumor cells. In contrast to targeting PD-1, targeting PD-L1 may not inhibit T-cell responses directed to tumors that constitutively express high levels of PD-L2. However, this appears to be uncommon in human cancer.

Similar to other tumor-targeting therapeutic antibodies (e.g., trastuzumab; ref. 30), optimal targeting of tumor PD-L1 will also likely require high levels of the therapeutic antibody to be administered to maintain PD-L1 saturation in tumors. In the case of CNS tumors or metastases, high

**Table 2.** Expression of PD-L1 in tumors

Cancer type	% PD-L1 <sup>+</sup>	References
Melanoma	40–100	Wang and colleagues (42) Hino and colleagues (43) Dong and colleagues (44)
Non-small cell lung cancer	35–95	Wang and colleagues (42) Dong and colleagues (44) Konishi and colleagues (45)
Nasopharyngeal cancer	68–100	Yang and colleagues (17) Hsu and colleagues (46)
Glioblastoma/mixed glioma	100	Wintterle and colleagues (47)
Colon adenocarcinoma	53	Dong and colleagues (44)
Hepatocellular carcinoma	45–93	Wang and colleagues (48) Gao and colleagues (49) Zeng and colleagues (50)
Urothelial cancer	28–100	Nakanishi and colleagues (51) Inman and colleagues (52)
Multiple myeloma	93	Liu and colleagues (53)
Ovarian cancer	33–80	Dong and colleagues (44) Hamanishi and colleagues (54) Brown and colleagues (55)
Gastric carcinoma	42	Wu and colleagues (56)
Esophageal cancer	42	Ohigashi and colleagues (57)
Pancreatic cancer	39	Nomi and colleagues (58)
RCC	15–24	Wang and colleagues (42) Krambeck and colleagues (59) Thompson and colleagues (60)
Breast cancer	31–34	Dijkers and colleagues (30) Ghebeh and colleagues (61)
Lymphomas	17–94 <sup>a</sup>	Xerri and colleagues (62) Wilcox and colleagues (63)
Leukemias	11–42	Tamura and colleagues (64) Berthon and colleagues (65) Kozako and colleagues (66) Chen and colleagues (67)

<sup>a</sup>Includes peripheral T-cell lymphomas, diffuse large cell lymphoma, or small lymphocytic lymphoma.

levels of therapy will also likely be required to penetrate the CNS (31).

One potential advantage of targeting PD-L1 compared with targeting PD-1 lies in leaving PD-L2, expressed only in the minority of human tumors, uninhibited. For organs such as the lung, this may reduce the likelihood of developing severe inflammatory toxicity (19, 26), which has been observed with clinical trials of a PD-1–directed therapeutic antibody (14% drug-related grade 3–4 adverse events, 3% drug-related pneumonitis, 1% drug-related pneumonitis resulting in death), but not with a PD-L1–directed therapeutic antibody (9% drug-related grade 3–4 adverse events, 0% drug-related pneumonitis; refs. 19, 26). In preclinical models, mice deficient in PD-L2 or in which PD-L2 is blocked exhibit hypersensitive T-helper (T<sub>H</sub>)<sub>2</sub> responses, exacerbated airway inflammation associated with elevated interleukin (IL)-4 levels, and attenuated

T<sub>H</sub>1 immunity (27). These results highlight the importance of PD-L2 in regulating T<sub>H</sub>2-mediated inflammation and are consistent with IL-4 being a primary driver of PD-L2 expression as a direct means to dampen T<sub>H</sub>2 responses (32). Because T<sub>H</sub>2 inflammation can promote tumor development, in part, through its promotion of alternatively activated macrophages, targeting PD-L1 may provide a means to preferentially enhance T<sub>H</sub>1 responses while allowing suppression of tumor-promoting T<sub>H</sub>2 responses. Moreover, targeting PD-L1 versus PD-1 provides the advantage of inhibiting additional PD–ligand interactions, such as that of PD-L1/B7.1, which appears to function uniquely to inhibit T-cell responses (16, 17). Additional potential advantages are suggested by *in vivo* studies that have found a greater antitumor effect of anti-PD-L1 blockade compared with anti-PD-1 or anti-PD-L2 blockade (33). Hence, targeting PD-L1, with a therapy



that inhibits all the interactions PD-L1 engages in, may further enhance the generation of the antitumor immune responses, an effect that would not be realized through restricted targeting of PD-1.

### Considerations for PD-L1 and PD-1 Cancer Therapies

A detailed knowledge of antibody structure and activity now allows us to engineer therapies to optimize biologic activity. The development of multiple monoclonal antibodies (mAb) that target the PD-L1/PD-1 pathway provide the potential to reveal quite distinct mechanisms of action, activity, and toxicity profiles. In addition to the distinctions between targeting PD-L1 and PD-1, therapies that can mediate antibody-dependent cell-mediated cytotoxicity (ADCC) may significantly change the effects of therapy (Table 1).

#### Fc domains may be determinant of PD-L1/PD-1-targeted effects

The majority of therapeutic mAbs currently being used to target cancer cells directly contain a human immunoglobulin G1 (IgG1) heavy chain. The data supporting the role of ADCC as a major mechanism of mAb activity are convincing (34). Studies with an antibody targeting lymphocytes in *in vitro* animal models and correlative clinical reports indicate that the interaction between mAb and Fc receptor (FcR) has a major contribution to the clinical antitumor activity (35, 36).

If the objective of PD-L1- or PD-1-targeted therapies is direct tumor killing of PD-L1- or PD-1-expressing tumors, ADCC function should be maximized. However, maximizing ADCC may compromise the antitumor immune-enhancing effects of PD-L1/PD-1 pathway inhibition. Because of expression of PD-L1 and PD-1 on activated T cells, which is often further elevated at the site of antigen exposure (i.e., tumor microenvironment; ref. 37), immune effector cells may be particularly susceptible to ADCC in the same fashion that normal B lymphocytes are rapidly cleared with administration of an IgG1 antibody-targeting CD20 such as rituximab (34). Such ADCC activity might also lead to killing of other PD-1- or PD-L1-expressing cells, such as dendritic cells or regulatory T cells, and its extent would be underrepresented in monitoring cell counts in blood because this activity may primarily occur in the tumor microenvironment. For these reasons, IgG1 Fc-based therapies are more likely to mediate their activity through direct killing of PD-1- or PD-L1-expressing cancer cells and killing of PD-1- or PD-L1-expressing regulatory cells rather than by immune enhancement through inhibition of the PD-L1/PD-1 axis. Such approaches have not yet been reported to result in significant monotherapy clinical responses in human solid tumors.

ADCC can be modulated with the use of an IgG4 antibody, which would likely reduce both the direct antitumor activity as well as the killing of PD-L1- or PD-1-expressing tumor-infiltrating lymphocytes. While IgG4 antibodies

exhibit reduced ADCC relative to IgG1, they may still retain significantly greater ADCC when compared with engineered antibodies that contain specific changes to more effectively impair Fc $\gamma$  receptor interactions (38). Hence, the characteristics of PD-L1/PD-1 pathway-targeted therapies can be described in terms of increasing direct ADCC-mediated antitumor activity and decreasing antitumor immune enhancement, and vice versa (Table 1).

#### Potential safety considerations for targeting PD-L1 and PD-1

While use of many immune-enhancing therapies, such as cytokine therapy (IL-2, IFNs) and more recently anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) therapy, are limited by their toxicity profiles (39, 40), preliminary reports of the safety and tolerability profile of PD-L1 and PD-1 inhibition are quite promising. Despite sharing a T-cell-based mechanism with anti-CTLA-4 antibodies, inhibition of PD-L1/PD-1 has been generally well tolerated and appears to be associated with less frequent immune-related adverse events than are seen with the former, such as colitis, hepatitis, and endocrinopathies (19, 28, 41). This difference is less surprising when we consider that PD-L1/PD-1 inhibition results primarily in disinhibiting existing chronic immune responses, rather than generating new, autoreactive T cells. For this reason, the toxicity of this class of agent may be more prominent in patients that have underlying autoimmunity that is controlled by PD-L1 and PD-L2. Uncommon immune and inflammatory adverse events have been reported, and future clinical studies will be necessary to further understand these potential toxicities.

#### PD-L1 tumor expression as a biomarker

PD-L1 appears to be broadly expressed on a wide variety of solid tumors and hematologic malignancies, and its expression has been reported to confer an unfavorable prognosis (Table 2; refs. 17, 30, 42–67). These findings support the hypothesis that PD-L1 expression is a highly conserved mechanism for cancer to escape immune-mediated destruction. Tumor expression of PD-L1 has been reported to be associated with positive responses to PD-L1/PD-1 pathway inhibition in clinical studies (19, 20, 26). Further clinical studies should address these associations.

Recent studies have ranked the prevalence of DNA mutations among various cancer types; heading the list of cancers bearing the highest mutation rate and complexity are melanoma, squamous cell carcinoma of the lung, and adenocarcinoma of the lung (38, 68, 69). Interestingly, this ranked prevalence of mutations bears a striking resemblance to the hierarchy of PD-L1 expression prevalence in those same human cancer types. Such correlations suggest that the degree of mutagenesis may correlate with the degree of immunogenicity of any given tumor. When we consider that any given mutation provides a chance to generate a neoantigen, which can be recognized as foreign by T cells, one can hypothesize that increasing tumor mutations may also increase immunogenicity. Immunogenic tumors are then either eradicated ("immune editing") by the immune

response (70) or develop mechanisms, such as expression of PD-L1, to evade immune destruction (11). Moreover, focal PD-L1 expression in melanoma tumors has also been spatially linked to regions of active T-cell inflammation, suggesting a causal role of T-cell-derived IFN- $\gamma$  in driving local PD-L1 expression in response to immune-mediated attack (3). PD-L1 expression in a given cancer would then reflect the degree of current tumor-specific T cells functionally engaged but restrained at the tumor site.

### Future Directions

The era of targeted therapies has focused on overexpressed cancer proteins that are synthetic lethal and critical for cancer cell survival. While many targeted therapies are highly active, it should be noted that cancer is highly mutagenic and highly adaptable. Targeted therapy is incapable of changing with cancer and, ultimately, a clone that circumvents the targeted therapy emerges or evolves (71). Cancer immunotherapy has the potential to address this. The host immune response is highly adaptable, as it necessarily evolved the capacity to control rapidly mutating viruses and, similarly, adapted to match the cancer's ability to mutate and evolve. Increased death of tumor cells is associated with more effective release and presentation of tumor antigens, enabling and expanding the breadth of the T-cell response. However, human cancer can present as a highly aggressive evolving disease, limiting opportunities to fully generate anticancer immune responses. Combining PD-L1/PD-1-targeted therapy with other anticancer treatment may provide time to generate these immune responses. Such approaches should be made in a rational fashion, as many cancer therapies can impact T-cell function, either positively or negatively (72). In addition, PD-L1/PD-1 inhibition alone does not appear to help all patients with cancer. While patient selection through the use of predictive biomarkers is a reasonable strategy, combination approaches may help address the reasons for failed or incomplete responses to PD-L1/PD-1 inhibition.

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Considering the steps required for generating an effective antitumor immune response, a combination that enhanced the immune priming, trafficking, and/or effector phases may improve clinical outcomes. Such approaches could include radiation therapy (73), antiangiogenic therapy (74–77), or adoptive cell transfer (78). Similarly, combining blockade of PD-L1/PD-1 with blockade of additional immune checkpoint inhibitors or inhibitory ligands may also improve responses, although caution is warranted given the potential to exacerbate autoimmunity. In addition, tumor-targeted agents (such as vemurafenib) that significantly reduce tumor burden but alone are insufficient to induce durable regression (17, 79) would provide ideal partners for immune therapy such as blockade of PD-L1/PD-1. Finally, effective tumor-targeted agents that additionally possess immune-enhancing properties could optimally combine with anti-PD-L1/PD-1 to generate durable and adaptable antitumor immunity (6, 75, 80).

### Disclosure of Potential Conflicts of Interest

F.S. Hodi has served as consultant/advisory board member for Genentech/Roche, Bristol-Myers Squibb, and Merck and has received clinical trial support from Bristol-Myers Squibb, Genentech/Roche, and Merck. No potential conflicts were disclosed by the other authors.

### Authors' Contributions

**Conception and design:** D.S. Chen, B.A. Irving, F.S. Hodi  
**Development of methodology:** D.S. Chen  
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** D.S. Chen  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** D.S. Chen, F.S. Hodi  
**Writing, review, and/or revision of the manuscript:** D.S. Chen, B.A. Irving, F.S. Hodi  
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** D.S. Chen, F.S. Hodi  
**Study supervision:** D.S. Chen

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