The emergence of programmed death-ligand 1 (PD-L1)/programmed death-1 (PD-1)–targeted therapy has demonstrated the importance of the PD-L1 : PD-1 interaction in inhibiting anticancer T-cell immunity in multiple human cancers, generating durable responses and extended overall survival. However, not all patients treated with PD-L1/PD-1–targeted therapy experience tumor shrinkage, durable responses, or prolonged survival. To extend such benefits to more cancer patients, it is necessary to understand why some patients experience primary or secondary immune escape, in which the immune response is incapable of eradicating all cancer cells. Understanding immune escape from PD-L1/PD-1–targeted therapy will be important to the development of rational immune-combination therapy and predictive diagnostics and to the identification of novel immune targets. Factors that likely relate to immune escape include the lack of strong cancer antigens or epitopes recognized by T cells, minimal activation of cancer-specific T cells, poor infiltration of T cells into tumors, downregulation of the major histocompatibility complex on cancer cells, and immunosuppressive factors and cells in the tumor microenvironment. Precisely identifying and understanding these mechanisms of immune escape in...
individual cancer patients will allow for personalized cancer immunotherapy, in which monotherapy and combination immunotherapy are chosen based on the presence of specific immune biology. This approach may enable treatment with immunotherapy without inducing immune escape, resulting in a larger proportion of patients obtaining clinical benefit.

**Key words:** cancer immunotherapy, personalized cancer immunotherapy, PD-1/PD-L1, immune escape, cancer-immunity cycle, resistance

**introduction**

The generation of anticancer immunity, which is mediated in large part by CD8+ cytotoxic T lymphocytes, is a multistep process termed the cancer-immunity cycle [1] (Figure 1).

The steps of the immunity cycle include the following: (1) release of cancer cell antigens, (2) cancer antigen presentation, (3) priming and activation, (4) trafficking of T cells to tumors, (5) infiltration of T cells into tumors, (6) recognition of cancer cells by T cells, and (7) killing of cancer cells. Numerous factors can help drive or suppress anticancer immunity at each step of the cancer-immunity cycle [1–4]. These include suppressive factors in the tumor microenvironment (TME), including the cell-associated factor programmed death-ligand 1 (PD-L1) and its interaction with one of its receptors, programmed death-1 (PD-1). Other immunosuppressive factors include soluble mediators that impair cancer antigen-presentation capabilities, thereby indirectly causing suboptimal T-cell priming and activation (steps 2 and 3), or inhibit expression of adhesion molecules on endothelial cells to impede T-cell infiltration (step 5). In the case of PD-L1, expression by tumor cells and tumor-infiltrating immune cells impairs cytolytic T-cell activity (step 7) [5–10]. In addition to PD-1, PD-L1 also binds to B7.1 expressed on activated antigen-presenting cells (APCs), which inhibits T-cell responses [11, 12].

Cancer immunotherapies that target the PD-L1 : PD-1 axis of immune regulation have demonstrated remarkable efficacy in a wide range of cancers. In addition to clinical activity in historically immunogenic cancers such as melanoma [13–19] and renal cell carcinoma (RCC) [20–23], anti-PD-L1 and anti-PD-1 agents can produce anticancer responses in a growing list of malignancies, including non-small-cell lung cancer (NSCLC) [9, 24–29], urothelial carcinoma [30–32], triple-negative breast cancer [33, 34], lymphoma [35–37], and head and neck cancer [38, 39]. Although these results highlight a key role for PD-L1 : PD-1 in suppressing anticancer immunity in multiple

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**Figure 1.** The cancer-immunity cycle. Figure modified from Chen and Mellman [1], with permission from Elsevier.
cancers, only a subset of patients with each cancer type respond to treatment and not all responders continue to respond indefinitely.

Biomarker studies conducted as a part of anti-PD-L1/PD-1 clinical trials have supported the hypothesis that these agents are most effective in patients who have pre-existing anticancer immunity. For any tumor type, individuals may present with cancer that is inflamed or noninflamed (excluded or immune deserts) [40]. However, certain tumor types appear to present more commonly with specific immune findings. Tumors such as melanoma, lung, kidney, and urothelial cancer often present as inflamed, whereas colorectal and pancreatic cancers often present as excluded and prostate cancer often presents as non-inflamed immune deserts [41–45]. Common characteristics of responding inflamed tumors include dense CD8+ T-cell infiltrates, a broad chemokine profile, PD-L1 expression on immune cells, a type 1 interferon (IFN) signature, and elevated expression of IFN-γ-induced genes [9, 40, 46]. On the basis of the biology of the PD-L1:PD-1 pathway, these characteristics of inflamed tumors are hypothesized to have a causal relationship, with the recognition of cancer-associated antigens by CD8+ T cells leading to the local production of IFN-γ, which ultimately induces the adaptive expression of PD-L1 on neighboring tumor-infiltrating immune cells and, in some cases, on tumor cells [47–49]. Because PD-L1 is expressed by antigen-experienced CD8+ T cells to limit T-cell receptor (TCR) signaling, PD-L1 expression can serve as a negative feedback regulator of cytolytic activity, acting through its receptors PD-1 and B7.1. Therefore, inflamed tumors are thought to have a pre-existing CD8+ T-cell response to their tumor, with the establishment of anticancer immunity kept in check by intratumoral PD-L1 expression (step 7).

The accumulation of mutations is a central part of the oncogenic process, which may contribute broadly to immunogenicity and potentially to the inflamed cancer phenotype [5]. Recent studies that examined the mutational landscape of inflamed tumors have supported this notion and demonstrated that T cells specific for neo-epitopes generated by nonsynonymous mutations may be responsible for establishing the inflamed phenotype [50–56]. High nonsynonymous mutation load is correlated with increased expression of genes encoding the cytolytic proteins granzyme A and perforin and T-cell–associated genes in multiple tumor types, including NSCLC, melanoma, and head and neck cancer [57]. Tumor-infiltrating T cells that recognize tumor-specific mutated antigens have been detected in these cancers, suggesting that increased mutational load augments tumor immunogenicity by activating neo-antigen–specific T cells [58–60]. However, there is evidence to suggest that factors beyond mutational load can contribute to response. A recent study identified a transcriptomic signature, termed the innate anti-PD-1 resistance signature, enriched in genes associated with mesenchymal transition, angiogenesis, hypoxia, and wound healing, that was overrepresented in metastatic melanoma anti-PD-1 nonresponding tumors [61]. In addition, responding tumors were enriched for mutations in the DNA repair gene BRCA2.

Types of cancer with a relatively higher frequency of nonsynonymous mutations—such as melanoma, NSCLC, bladder, and head and neck cancer [62, 63]—may be particularly responsive to PD-L1:PD-1 pathway blockade [9, 13, 15, 16, 24, 26, 29, 32, 38, 39]. In addition, although colorectal cancer (CRC) has been reported to be associated with a lower monotherapy response rate to PD-L1/PD-1 inhibitors, higher response rates have been observed in patients with CRC who had elevated mutation loads due to deficiency in DNA mismatch-repair enzymes versus patients with mismatch-repair proficient CRC [64]. Together, these associations between clinical efficacy and nonsynonymous mutation load suggest that neo-antigen–specific T cells are the targets of PD-L1:PD-1 pathway blockade. In support of this, responsiveness to PD-L1/PD-1 blockade correlated with the expansion and increased effector functions of T cells specific for a mutated tumor antigen in NSCLC [65]. Similarly, in a murine fibrosarcoma model, the efficacy of anti-PD-1 therapy was associated with increased intratumoral CD8+ T cells that recognize a tumor-specific neo-antigen [66]. Furthermore, neo-antigen–specific CD8+ T cells are enriched in the PD-1–positive fraction of tumor-infiltrating lymphocytes compared with their PD-1–negative counterparts in melanoma [67], suggesting a stronger activation for the neo-antigen–specific T cells.

In contrast with inflamed tumors, noninflamed tumors lack the CD8+ T-cell infiltrate, broad chemokine profile, and adaptive expression of immune inhibitory molecules such as PD-L1 [40, 41]. The underlying cause of a noninflamed phenotype in some patients may be a lack of acceptably immunogenic antigens that are intrinsic to the tumor. This phenomenon is also known as immunologic ignorance and is distinct from active immune escape mechanisms that may contribute to a noninflamed tumor environment. Alternatively, tumors with a significant nonsynonymous mutation load may display a noninflamed phenotype due to active immune escape mechanisms that cause a defect in one or more of steps 1–5 of the cancer-immunity cycle. In addition, anticancer responses with a defect in the early stages of the cancer-immunity cycle are expected to result in the same noninflamed immune contexture as ignorant tumors. The development of biomarkers that can identify the underlying cause of a noninflamed TME may help guide therapeutic strategies to overcome immunologic ignorance and/or immune escape.

**tumor immune escape**

Tumor escape from cancer immunotherapy differs from traditional drug resistance. For most anticancer therapies, such as chemotherapy or targeted therapy, resistance to treatment can be mediated by the expression of specific proteins such as drug efflux pumps that remove toxic reagents from the tumor cells, genomic editing to induce loss of expression of the therapeutic target, or upregulation of compensatory signaling pathways. Unlike tumor-directed therapies that use static mechanisms such as chemotherapy, cancer immunotherapies—including PD-L1:PD-1 blockade—are hypothesized to potentiate a polyclonal anticancer T-cell response that is capable of evolving and overcoming tumor-derived resistance mechanisms.

**primary versus secondary escape.** Primary escape occurs when patients who have not previously received a given treatment fail to respond, and secondary escape occurs when patients who have responded to a treatment stop responding. Little data regarding mechanisms of secondary escape to anti-PD-L1/PD-1
therapy are available, and it is unclear whether the mechanisms of secondary escape are distinct from those of primary escape. Primary escape from anti-PD-L1/PD-1 therapy may be mediated by the numerous immunosuppressive mechanisms that inhibit anticancer T-cell responses. Owing to differences in the immune contexture at baseline, the mechanisms that govern primary escape in inflamed tumors are predicted to be fundamentally different from those in noninflamed tumors. Escape in noninflamed tumors may be a consequence of a lack of immunogenic cancer antigens, suboptimal T-cell activation, or T-cell trafficking. The question of escape from anti-PD-L1/PD-1 therapies has not been comprehensively explored. Here, we used the framework of the cancer-immunity cycle to address the following questions: (i) what are the primary escape mechanisms in patients with either inflamed or noninflamed tumors that fail to respond to anti-PD-1 and anti-PD-L1 therapies? and (ii) why do some patients with an initial response stop responding (secondary escape)?

**escape in noninflamed tumors**

The successful passage through the early stages of T-cell priming and activation (steps 1–3) and infiltration of T cells into tumors (steps 4 and 5) is a prerequisite for an inflamed tumor phenotype. Because PD-L1 : PD-1 blockade is primarily active in patients with inflamed tumors, nonresponsive patients with noninflamed tumors are likely to have a defect in the early stages of the cancer-immunity cycle. Therefore, escape mechanisms that impair T-cell priming and trafficking to tumor tissues are hypothesized to be the cause of nonresponsiveness to anti-PD-L1 or anti-PD-1 treatment in noninflamed tumors.

**T-cell priming and activation (cycle steps 1–3)**

*escape due to poor tumor immunogenicity (step 1).* The cancer-immunity cycle is initiated by the release of cancer cell antigens from dying tumor cells, which are taken up by APCs, such as dendritic cells (DCs). Following antigen uptake, DCs migrate to the draining lymph node to present processed tumor-associated peptides in the context of major histocompatibility complex class I (MHC-I) molecules to CD8+ T cells. Tumor-associated CD8+ T cells recognize self-antigens such as tissue differentiation antigens or peptides derived from overexpressed tumor proteins or altered self-proteins generated by mutated proteins that arise from the oncogenic process [68]. Compared with self-reactive T cells that are subject to negative selection during thymic development, T-cell clones that recognize mutated self-proteins or neo-antigens are thought to provide higher-quality anticancer responses due to higher-affinity TCRs and increased precursor frequency [51, 69, 70]. Given the correlation between mutation burden and the responses to PD-L1 : PD-1 pathway inhibition, reduced nonsynonymous mutation burden may be the underlying reason for the lack of tumor immunogenicity in some nonresponsive patients. Additionally, tumor immunogenicity may also be driven by the quality of the tumor-associated antigens expressed in the context of MHC molecules. Predicting the immunogenicity of tumor-associated antigens can be achieved through peptide–MHC-binding algorithms and in vitro testing of candidate antigens with autologous T cells [71, 72]. Another approach is the combination of whole-exome and transcriptome sequencing analysis with mass spectrometry of peptides eluted from MHC molecules [73]. These ongoing efforts to refine prediction pipelines may reveal the principal determinants of tumor immunogenicity and aid in cancer vaccine development.

Mechanisms of secondary escape include the loss of immunogenic antigens and the selection of cancer cell clones lacking antigens recognized by T cells. During tumor establishment, cancer immunosurveillance may eliminate cancer cell clones that express strongly immunogenic neo-antigens [74]. Cancer cell clones that escape immune selection are hypothesized to harbor fewer immunogenic antigens. Thus, secondary escape variants from PD-L1 : PD-1 blockade may be derived from cancer cell clones that do not express immunodominant epitopes recognized by T cells.

*escape due to reduced DC maturation (step 2).* DCs acquire the capacity to migrate to the draining lymph node, present processed antigens to T cells, and express costimulatory ligands for T cells through a process known as DC maturation [75]. In cancer, damage-associated molecular pattern (DAMP) molecules such as adenosine triphosphate (ATP) or high-mobility group box 1 (HMGB1), which are released by dying tumor cells, can induce DC maturation. Therefore, cancer cell death (step 1) is directly coupled with the subsequent antigen-presentation step by providing the stimuli for DC maturation. To prevent the initiation of the cancer-immunity cycle, tumors can inhibit DC maturation by functionally inactivating DAMP activity. For instance, oxidative posttranslational modifications of HMGB1 can abrogate its DC stimulatory activity, and enzymatic processing of ATP to its nonstimulatory products adenosine monophosphate (AMP) and adenosine can serve as escape mechanisms to prevent DC maturation [76, 77].

Numerous cancer-derived soluble factors inhibit DC maturation and antigen-presentation function, interleukin (IL) 10, macrophage colony-stimulating factor, vascular endothelial growth factor (VEGF), prostaglandins, transforming growth factor β (TGF-β), adenosine, and indoleamine 2,3-dioxygenase (IDO) [3, 78–80]. Suppressive immune cell subsets in the TME, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), also express inhibitory factors that impair DC maturation. By inhibiting DC maturation, these immunosuppressive molecules ultimately impair T-cell expansion and differentiation into IFN-γ-producing cells by reducing the expression of MHC and costimulatory molecules, which reduces the production of inflammatory cytokines such as IL-12, and by catabolizing nutrients required for T-cell activation.

*escape due to suboptimal T-cell activation (step 3).* An important consequence of DC maturation is the upregulation of ligands for costimulatory molecules. In addition to cognate antigen recognition, optimal T-cell activation requires costimulatory signals to promote T-cell proliferation, differentiation, survival, cytotoxic function, memory formation, and cytokine production [81]. Costimulatory interactions between DCs and T cells include B7.1/B7.2:CD28, 4-1BB:4-1BB, OX40:OX40, CD70:CD27, and GITRL:GITR [glucocorticoid-induced tumor necrosis factor-related gene (GITR) ligand (GITRL)]. In tumor tissues, DAMP-induced expression of costimulatory ligands is opposed by multiple
immunosuppressive factors, which leads to lower levels of costimulatory ligands and MHC molecules [3, 82, 83]. In the absence of appropriate costimulation, TCR activation leads to excessive calcium/nuclear factor of activated T-cell signaling and the subsequent expression of negative regulatory factors that result in T-cell anergy or functional unresponsiveness [84]. Defective T-cell costimulation can be overcome by using stimulatory antibodies specific for costimulatory molecules. Indeed, agonistic anti-4-1BB, anti-OX40, or anti-GITR antibodies combine with PD-L1:PD-1 inhibition in preclinical tumor models to increase anticancer-immune activity, suggesting that suboptimal costimulation during T-cell priming can be a barrier for PD-L1:PD-1 blockade [85–87].

Partial T-cell activation can also result in limited production of IL-2 [88], an autocrine growth factor for T cells, leading to impaired CD8+ T-cell responses [89–93]. Preclinically, low-dose IL-2 administration augments anti-PD-L1-induced responses to chronic viral infection [94]. Furthermore, high-dose IL-2 immunotherapy has demonstrated efficacy in some patients with metastatic melanoma and RCC, and is approved for metastatic melanoma (aldesleukin; Proleukin) [95]. Novel IL-2 variants that augment T-cell activation but minimize endothelial involvement may provide safer methods to activate tumor-specific T cells [96, 97]. Additionally, tumor-targeted immunocytokines that contain an IL-2 variant fused to tumor-specific antibodies are in development, including anti-carcinobromic antigen IL-2v and anti-fibroblast activation protein IL-2v [98, 99].

During T-cell priming, T cells express negative feedback regulators, such as cytotoxic T-lymphyocyte–associated protein 4 (CTLA-4), to limit T-cell activation. Consistent with its role in T-cell priming, treatment with blocking antibodies specific for CTLA-4 induces T-cell expansion and broadening of the peripheral T-cell repertoire [100, 101]. Increased overall survival was observed in patients with melanoma treated with anti-CTLA-4 antibodies, which supports initial observations made in preclinical models [102]. Therefore, T-cell checkpoints that limit T-cell activation in step 2 of the cancer-immunity cycle may, in some cases, serve as primary escape mechanisms from PD-L1:PD-1 blockade.

In patients with an early defect in the cancer-immunity cycle, combination immunotherapy may be the best approach. Combining a PD-L1 or PD-1 inhibitor with an agent that acts at another cycle step may convert a noninflamed tumor into an inflamed tumor and result in improved responses. For example, anti-CTLA-4 and anti-PD-1 antibodies given in combination to patients with melanoma increased progression-free survival compared with each monotherapy [103]. Immune toxicity was also enhanced compared with monotherapy treatment. Inhibition of CTLA-4 may lead to greater noncancer-specific T-cell activation, which is further enhanced by PD-L1:PD-1 blockade. Such findings highlight the potential power of combination immunotherapy, while also highlighting the need to identify therapies that are as specific as possible in enhancing anticancer immunity without enhancing immunity directed against normal tissues.

**T-cell trafficking and infiltration (cycle steps 4 and 5)**

T-cell priming in the draining lymph node can lead to the clonal expansion of tumor-specific T cells and the expression of cell adhesion molecules and chemokine receptors required for infiltration into inflamed tissues. Therefore, T-cell activation (step 3) is critical for equipping T cells with surface receptors required for T-cell trafficking and tumor infiltration. Following T-cell priming, T cells egress the lymph node and enter the circulation to migrate to inflamed tissues [104]. Activated T cells extravasate from the blood in a multistep adhesion process [105]. Extravasation requires tethering and rolling along the endothelium, which is mediated by adhesion molecules, exposure to a chemoattractant stimulus provided by chemokines and G-protein–coupled receptors, and arrest mediated by activated integrins (Figure 2).

**escape due to impaired T-cell trafficking and infiltration (steps 4 and 5).** During T-cell activation, the local cytokine environment dictates the chemokine receptors expressed on T cells. IFN-γ induces the expression of CXCR3, a chemokine receptor that is highly expressed by tumor-infiltrating lymphocytes in human cancers [106]. Although the chemokine:chemokine receptor system has a high degree of redundancy, preclinical studies suggest that CXCR3 is required for T-cell migration into mouse tumors [107]. Interestingly, increased expression of CXCL9, a ligand for CXCR3, is also regulated by IFNs and is associated with the inflamed tumor phenotype [9]. Therefore, low levels of CXCL9 or other CXCR3 ligands, such as CXCL10 and CXCL11, are hypothesized to contribute to reduced CD8+ T-cell infiltration and primary escape at steps 4 and 5. CXCR3 ligands are further regulated by posttranslational modifications and proteolysis [108–110]. Furthermore, cleaved CXCR3 ligands can serve as receptor antagonists [109]. In mouse tumor models, chemokine cleavage impairs CXCR3-mediated T-cell trafficking and antitumor activity [111] (Figure 2).

Tumors can also use various mechanisms that modify the local endothelium to inhibit T cells from infiltrating the tumor site, including downregulation of adhesion molecules that are essential for T-cell extravasation [43]. In particular, angiogenic factors (e.g. VEGF) produced in the TME inhibit T-cell adhesion to the endothelium by downregulating adhesion molecules, including intercellular adhesion molecule 1, intercellular adhesion molecule 2, and vascular cell adhesion molecule 1 on endothelial cells [112–114]. Consistent with this, increased levels of VEGF within tumors have been associated with an absence of intratumoral T cells [115], and inhibition of VEGF:VEGF receptor-2 interactions can increase infiltration [116]. Together with the immunosuppressive factors IL-10 and prostaglandin E2, VEGF also induces the expression of Fas ligand, which induces cell death in tumor-infiltrating CD8+ T cells [117]. Another mechanism by which tumors may inhibit T-cell infiltration is the upregulation of endothelin-B receptor by tumor endothelial cells, which has been associated with an absence of tumor-infiltrating T cells in multiple cancer types [118] (Figure 2).

**escape due to stroma-dependent exclusion.** Even after successfully infiltrating tumor tissues, CD8+ T cells can be retained in the tumor margins and excluded from the tumor center [115, 119–121]. Immunosuppressive leukocytes and cancer-associated fibroblasts (CAFs) that reside in the tumor margins can physically exclude T cells via the production of extracellular...
matrix proteins or actively prevent T-cell migration to the tumor center through chemokine-mediated repulsion and other unidentified mechanisms [43, 122]. An imaging study of human lung tumors revealed that areas of dense accumulation of fibronectin or collagen in the tumor stroma prevented T cells from contacting tumor cells [123]. In addition to producing extracellular matrix, CAFs also produce CXCL12, which has been shown to inhibit intratumoral T-cell infiltration in a pancreatic cancer model [124]. In multiple preclinical models, CAF depletion by targeting fibroblast activation protein inhibits tumor growth in a lymphocyte-dependent manner [125–127], and inhibition of CXCR4—the receptor for CXCL12—has been shown to increase intratumoral T-cell accumulation [124]. In human tumors, an excluded CD8+ T-cell infiltrate was one of the phenotypes observed in patients who did not respond to anti-PD-L1 therapy [9]. Future studies aimed at comparing the immune contexture of CD8+ T-cell–excluded tumors and CD8+ T-cell–containing inflamed tumors may reveal escape mechanisms used by stromal cells.

**escape in inflamed tumors**

Similar to the initiating steps of the cancer-immunity cycle, the final steps take place in the TME and are subject to the same sources of immunosuppression. Soluble factors such as IL-10, VEGF, prostaglandins, TGF-β, adenosine, and IDO have the dual role of inhibiting T-cell priming as well as cytolytic T-cell activity. In preclinical models, combining agents that block adenosine or IDO signaling with PD-L1:PD-1 inhibition have demonstrated additive antitumor activity [128–130]. Although inflamed tumors may not have a primary defect in T-cell priming, the role of adenosine or IDO signaling inhibitors in augmenting T-cell activation may contribute to combination activity.

Because the PD-L1 : PD-1 axis is only one of many sources of immunosuppression, it is not surprising that some patients fail to respond to anti-PD-L1/PD-1 treatment despite harboring an inflamed TME. Even in patients who initially responded to PD-L1 : PD-1 inhibition, reinforced immunosuppression as a reaction to T-cell–dependent attack can potentially induce secondary escape.

**tumor cell recognition and killing (cycle steps 6 and 7)**

Successful progression through the first five steps of the cancer-immunity cycle is predicted to generate an inflamed TME. Therefore, escape mechanisms that target the tumor cell...
recognition and killing steps are hypothesized to apply to inflamed tumors. CD8+ T-cell–mediated killing of cancer cells requires TCR recognition of cognate peptide–MHC-I complexes on cancer cells. CD8+ T cells can kill target cells via two mechanisms: the granule–exocytosis pathway, which is mediated by perforin and granzymes [131], and death receptor–ligand interactions, such as FasL: Fas [132]. Tumor cell killing is predicted to release additional cancer antigens to further propagate the cancer-immunity cycle.

escape due to reduced recognition by immune cells (step 6). One mechanism by which cancer cells can evade T-cell recognition is via the loss, downregulation, or alteration of the MHC-I protein on the surface of cancer cells. This can be achieved by directly targeting MHC-I genes or proteins or indirectly by inhibiting the components of the peptide–MHC processing machinery in cancer cells. The loss of expression of cell-surface peptide–MHC-I can occur through the loss of expression of antigen, proteasome components, TAP1/TAP2, MHC-I, or β2-microglobulin, which can result from selective pressure from mutation, genetic loss, transcriptional repression, or epigenetic silencing of expression [133–135]. Recent cancer genome studies support somatic mutation of human leukocyte antigen (HLA) genes as a common mechanism of immune evasion [62, 136, 137]. Downregulation of MHC-I has been reported in a variety of cancers: the frequency of downregulation in primary lesions ranged from 16% to 50% [138], and MHC-I downregulation has been associated with disease progression and worse outcomes [139].

Secondary escape may also be due to the selection of cancer clones that harbor loss-of-function mutations in the MHC-I machinery. A recent study found an association between HLA mutations and gene expression signatures of effector lymphocytes in several tumor types, suggesting that the mutations arose in response to immune attack [136].

Although reduced MHC-I expression impairs cancer cell recognition by T cells, natural killer (NK) cells are able to mount an immune response against aberrant cells that are not displaying appropriate levels of MHC-I on their cell surface [140]. Thus, immune escape by MHC-I downregulation exposes cancer cells to NK cell-mediated immune surveillance. However, cancer cells can escape NK cell-mediated cytolysis by cleaving or releasing ligands for NKG2D, which is an activating receptor on NK cells [141, 142]. NKG2D ligand release may work in concert with MHC-I downregulation to allow tumors to escape both NK-cell and T-cell detection.

escape due to additive checkpoints (step 7). Tumor-infiltrating CD8+ T cells can coexpress multiple coinhibitory receptors in addition to PD-1, including B- and T-lymphocyte attenuator, lymphocyte-activation gene 3 protein (LAG-3), T-cell immunoglobulin domain, mucin domain-3 (TIM-3), and the recently described T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) [1, 48, 143]. Data from chronic viral infection models revealed that T-cell dysfunction induced by chronic TCR signaling is compounded by the expression of co-inhibitory receptors, such as LAG-3, TIM-3, and TIGIT [143–145]. PD-1+ CD8+ T cells expressing successively increasing numbers of inhibitory receptors were less capable of producing inflammatory cytokines and eliminating virally infected cells [144, 146]. Similarly, tumor-associated T cells that expressed additional coinhibitory receptors displayed a more severely exhausted phenotype and were less sensitive to PD-L1 : PD-1 blockade [147, 148]. Thus, escape from PD-L1 : PD-1 blockade could be achieved by additive expression of co-inhibitory receptors on CD8+ T cells. Combination immune therapy comprising anti-PD-L1/PD-1 antibodies with blocking antibodies to LAG-3, TIM-3, or TIGIT resulted in enhanced antitumor responses compared with single agents in preclinical models [143, 149–151]. Interestingly, blocking LAG-3, TIM-3, or TIGIT alone had a minimal impact on tumor growth inhibition, but was active only in combination with anti-PD-L1/anti-PD-1 treatment, suggesting that the suppressive capacity of the PD-L1 : PD-1 axis is dominant over other known co-inhibitory receptors. The combination of an anti-PD-1 antibody (nivolumab) and an anti-LAG-3 antibody is being evaluated in a phase 2 study of patients with NSCLC (NCT02750514).

Interestingly, PD-L2—an alternate ligand for PD-1—does not appear to confer clinically meaningful suppression of anticancer T cells in human tumors because therapeutics designed to inhibit PD-L1 : PD-1 interactions (e.g. PD-L1–targeted therapies) appear to have clinical activity similar to that of therapeutics designed to inhibit PD-L2 : PD-1 interactions (e.g. PD-1–targeted therapies) [16, 18, 19, 27–29]. Expression of PD-L1 and PD-L2 in human tumors has been associated with a higher response rate to atezolizumab (a PD-L1 inhibitor) when compared with tumors that express PD-L1 but not PD-L2 [9]. Furthermore, gene expression from a randomized study in NSCLC with atezolizumab demonstrated a numerically greater survival benefit for patients selected for a higher expression of PD-L2 when compared with PD-L1 [152]. The role of PD-L2 in suppressing anticancer immunity in humans remains poorly understood.

The upregulation of alternative inhibitory proteins following treatment with PD-L1/PD-1 inhibitors may provide a mechanism of secondary escape at this step. In fact, TIM-3 upregulation has been demonstrated in anti-PD-1 adaptive resistant tumors [153].

escape due to immunosuppressive cells (step 7). Immune cells present in tumors constitute a significant source of immune suppression. The TME promotes the polarization of tumor-associated macrophages into tumor-supporting M2-like macrophages, which produce IL-10 instead of IL-12, thereby suppressing CD8+ T-cell responses [154, 155]. A high density of tumor-associated macrophages has been associated with poor prognosis in some cancers [156]. M2DCs are a heterogeneous group of cells that can potently suppress effector T-cell responses and induce Tregs [4, 155, 157]. These cells mediate their suppressive effects via the production of arginase, inducible nitric oxide synthase, and TGF-β [158–161]. Among the multiple activities of TGF-β relevant for inflamed tumors, TGF-β suppresses cytotoxic T-cell and NK-cell function by inhibiting expression of the cytotoxic apparatus, including perforin and granzymes [142, 143]. IDO, which can be produced by cancer cells or immune cells, potently suppresses T-cell effector functions while promoting the generation and
activity of Tregs and MDSCs [162, 163]. Prostaglandin E₂, arginase, and adenosine also inhibit T-cell responses in the TME [164–166]. Adenosine, which is generated by the conversion of extracellular ATP through the enzymatic activity of the ectonucleotidases CD39 and CD73, acts to inhibit T-cell activation and expansion via the A₂A adenosine receptor [167]. Loss of CD73 expression in either cancer or host cells leads to tumor growth inhibition in preclinical models, indicating a nonredundant role for ATP conversion for immune escape [168, 169]. Antibodies that inhibit CD73 function augment anti-PD-1 activity in mouse tumor models, suggesting that ATP inactivation may contribute to escape from PD-L1 : PD-1 pathway inhibition [170]. In patients with bladder cancer, baseline expression of myeloid genes (IL-1β, IL-8, and CCL2) was lower in anti-PD-L1–responsive patients than in those who had stable or progressive disease, supporting a role for myeloid suppressor cells in cancer-immune escape [171].

Many tumors have a significant accumulation of Tregs, which can inhibit CD8⁺ T-cell responses and promote tumor progression. The accumulation of Tregs has been associated with a poor prognosis in many cancers [172–176]. Treg depletion in mouse tumor models has been shown to potentiate anticancer-immune responses, highlighting their nonredundant role in suppressing anticaner immunity [177–181]. Indeed, increased intratumoral CD8⁺/Treg cell ratios serve as a biomarker for productive T-cell responses to therapy. In mouse tumor models, transient Treg depletion synergizes with PD-L1 inhibition [150, 182, 183], suggesting that selective Treg depletion, or impairment of Treg suppressive activity, may combine in the clinic. Antibodies that target CTLA-4, GITR, and OX40 are associated with the preferential depletion of tumor-associated Tregs [184–187], suggesting that the observed synergistic effects of anti-PD-L1/ PD-1 agents and antibodies that target these molecules [103] may be in part due to transient Treg depletion. An increased frequency of Tregs at 12 weeks associated with disease progression in patients with melanoma treated with anti-PD-1 [14], and a higher ratio of effector T cells to Tregs was associated with response to anti-PD-L1 in patients with RCC [23]. It is likely that the contribution of Tregs to immune escape varies by tumor type and the TME, and their suppressive effects may be overcome by effector T-cell responses in some tumors. Even in patients who initially responded to PD-L1 : PD-1 inhibition, an increase in suppressive cells may reinforce immunosuppression as a reaction to T-cell–dependent attack and may induce secondary immune escape.

**Figure 3.** Personalized cancer immunotherapy paradigm. A wide range of cancer-immune phenotypes can exist, each with the potential to benefit from specific and distinct therapeutic strategies. In this proposed hypothetical algorithm, each patient’s tumor is characterized for biomarkers associated with the cancer-immunity cycle, including the presence of Th1 immunity, PD-L1 expression, exclusion of T cells from the tumor, MHC-I expression, and the presence or absence of other immune inhibitory factors and cells. Patients are then mapped to specific immunotherapy regimens that address the underlying cause of an ineffective anticaner-immune response. Patients with cancers that display evidence of pre-existing immunity, an inflamed phenotype (including strong PD-L1 expression), and a high mutational burden may require only monotherapy with anti-PD-L1 or anti-PD-1. Inflamed tumors expressing other immunosuppressive factors may require combination therapy, for example an IDO inhibitor plus anti-PD-L1/ PD-1. Patients with noninflamed tumors may benefit from a combination of an anti-PD-L1/ PD-1 agent plus a therapy that targets a proximal step of the cancer-immunity cycle, such as an anti-angiogenic agent, anti-OX40, or chemotherapy. Personalized cancer immunotherapy will enable the use of specific regimens precisely chosen to reflect the cancer-immune biology present in a given patient. CAR-T, chimeric antigen receptor T cells; CSF1R, colony-stimulating factor 1 receptor; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; MHC, major histocompatibility complex; PD-L1, programmed death-ligand 1.
toward a personalized cancer immunotherapy paradigm

Anti-PD-L1 and anti-PD-1 therapies have shown promising single-agent activity, leading to survival benefits in unselected patients and durable responses in a subset of patients with metastatic cancer. Indeed, some cancer patients may require only monotherapy inhibition of PD-L1:PD-1 to achieve immune eradication of their cancer. However, in other patients, a combination approach that targets the different potential primary and secondary immune escape mechanisms may be required. Treatment strategies that combine an anti-PD-L1 or anti-PD-1 agent with a therapy or therapies that target proximal steps of the cancer-immunity cycle are an attractive treatment paradigm, particularly in patients who have cancers that may not demonstrate evidence of strong pre-existing anticancer immunity and an inflamed phenotype. In patients who already demonstrate an inflamed phenotype, targeting other immunosuppressive factors in the TME may be critical to generating durable responses.

A wealth of clinical and biological data can be expected from the combination clinical trials being planned and conducted. These trials will hopefully provide proof-of-principle of enhanced efficacy and tolerability of combination immunotherapy and further elucidate the complex biology of the cancer-immunity cycle. With so many possibilities for primary and secondary escape mechanisms to cancer immunotherapy agents, it will be important for these trials to include a careful analysis of a wide range of pharmacodynamic, biological, and potentially predictive biomarkers. To this end, obtaining pretreatment and on-treatment tumor biopsy samples will be critical. With this information, clinicians will be better able to provide individual patients with the immunotherapy monotherapy or combination most likely to achieve a durable response. By combining an improved understanding of human cancer-immune biology and escape with further developed immune biomarker platforms and new immune modulators, the observed activity of cancer immunotherapies may be greatly enhanced.

We refer here to the selection of specific cancer immunotherapy regimens precisely chosen to reflect individual cancer-immune biology present in a given patient as ‘personalized cancer immunotherapy’. Proposed is a rational and hypothetical algorithm for personalized cancer immunotherapy (Figure 3). In this algorithm, each patient’s tumor is assessed for the presence or absence of a number of immune-related biomarkers, including PD-L1, and the presence and location of T cells. Understanding the biology within an individual patient may allow the mapping of individual tumors to where they exist biologically within the cancer-immunity cycle. This knowledge may enable treating physicians to specifically deliver the optimal immunotherapy or combination immunotherapy matched for the underlying immune biology present. For example, high PD-L1 expression, high mutational burden, and strong expression of IFN-γ, CD8, and CXCL9 may indicate that steps 1–6 of the cycle are likely to be intact in that patient, and treatment with a PD-L1 or PD-1 inhibitor may be all that is required to generate a durable response. However, PD-L1:PD-1 inhibition may not be sufficient in patients with low to no expression of PD-L1 in the TME, especially those with low infiltration of T cells [41] and little to no expression of Th1 effector genes. These patients may have a defect at a proximal cycle step, and combination therapy may be required to promote tumor immune cell activation and infiltration. In-depth knowledge of immunogenic antigens for recognition, activation of anticancer T cells, factors that influence tumor infiltration, and soluble and cell-associated immune suppressors will likely enable a greater sophistication in treatment selection and an increase in our ability to achieve immune eradication of cancer.

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