Immunity
Review

Oncology Meets Immunology: The Cancer-Immunity Cycle

Daniel S. Chen[1,3] and Ira Mellman[2,3,*]
[1]Stanford Medical Oncology, Stanford University School of Medicine, Stanford, CA 94305, USA
[2]Department of Biochemistry & Biophysics, University of California, San Francisco School of Medicine, San Francisco, CA 94143, USA
[3]Genentech, 1 DNA Way, South San Francisco, CA 94080, USA
*Correspondence: mellman.ira@gene.com
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The genetic and cellular alterations that define cancer provide the immune system with the means to generate T cell responses that recognize and eradicate cancer cells. However, elimination of cancer by T cells is only one step in the Cancer-Immunity Cycle, which manages the delicate balance between the recognition of nonself and the prevention of autoimmunity. Identification of cancer cell T cell inhibitory signals, including PD-L1, has prompted the development of a new class of cancer immunotherapy that specifically hinders immune effector inhibition, reinvigorating and potentially expanding preexisting anticancer immune responses. The presence of suppressive factors in the tumor microenvironment may explain the limited activity observed with previous immune-based therapies and why these therapies may be more effective in combination with agents that target other steps of the cycle. Emerging clinical data suggest that cancer immunotherapy is likely to become a key part of the clinical management of cancer.

Introduction

The development of cancer immunotherapy has reached an important inflection point in the history of cancer therapy (reviewed in Mellman et al., 2011). Durable monotherapy responses are consistently being reported for a broad range of human cancers with several different agents (Hamid et al., 2013a; Herbst et al., 2013; Hodi et al., 2010; Topalian et al., 2012b), providing a compelling argument that cancer immunotherapy is active in a range of indications beyond melanoma, a disease often thought to be atypically immunogenic (Jacobs et al., 2012). In addition to encouraging activity, many of the cancer immunotherapy approaches report safety profiles that are milder and more manageable than traditional or targeted (i.e., oncogene-centric) cancer therapies.

Cancer is characterized by the accumulation of a variable number of genetic alterations and the loss of normal cellular regulatory processes (Tian et al., 2011). These events have long been known to result in the expression of neoantigens, differentiation antigens, or cancer testis antigens, which can lead to presentation of peptides bound to major histocompatibility class I (MHCI) molecules on the surface of cancer cells, distinguishing them from their normal counterparts. Since the work of Boon and colleagues, we have known that these cancer-specific peptide-MHCI complexes can be recognized by $CD8^+$ T cells produced spontaneously in cancer patients (Boon et al., 1994). However, even when T cell responses occurred, they rarely provided protective immunity nor could they be mobilized to provide a basis for therapy.

As demonstrated by elegant analyses of cancer in mice, the continued deletion of cancer cells expressing T cell targets (immune editing) may enable cancers to evolve to avoid attack (Dunn et al., 2002). Despite these findings, recent results from human cancer have demonstrated that overcoming negative regulators to T cell responses in lymphoid organs (checkpoints) and in the tumor bed (immunostat function) are likely to explain the failure of immune protection in many patients (Mullard, 2013). Factors in the tumor microenvironment can act to modulate the existing activated antitumor T cell immune response, acting as an immune rheostat or "immunostat." This class of molecules, including PD-L1:PD-1 (reviewed in Chen et al., 2012; Topalian et al., 2012a), emphasizes that the immune response in cancer reflects a series of carefully regulated events that may be optimally addressed not singly but as a group. The challenge now is to use this new understanding to develop new drugs and implement clinical strategies.

The articles contained in this issue each address key aspects of how the immune response can control or be manipulated to enhance anticancer immunity (Galon et al., 2013; Kalos and June, 2013; Motz and Coukos, 2013; Palucka and Banchereau, 2013; van den Boorn and Hartmann, 2013; Zitvogel et al., 2013). Here, we will integrate this information and consider how it might best be used in clinical development.

The Cancer-Immunity Cycle

For an anticancer immune response to lead to effective killing of cancer cells, a series of stepwise events must be initiated and allowed to proceed and expand iteratively. We refer to these steps as the Cancer-Immunity Cycle (Figure 1). In the first step, neoantigens created by oncogenesis are released and captured by dendritic cells (DCs) for processing (step 1). In order for this step to yield an anticancer T cell response, it must be accompanied by signals that specify immunity lest peripheral tolerance to the tumor antigens be induced. Such immunogenic signals might include proinflammatory cytokines and factors released by dying tumor cells or by the gut microbiota (Figure 2, Table 1). Next, DCs present the captured antigens on MHCI and MHCII molecules to T cells (step 2), resulting in the priming and activation of effector T cell responses against the cancer-specific antigens (step 3) that are viewed as foreign or against which central tolerance has been incomplete. The nature of the immune response is determined at this stage, with a critical balance representing the ratio of T effector cells versus T regulatory cells being key to the final outcome. Finally, the activated effector T cells traffic

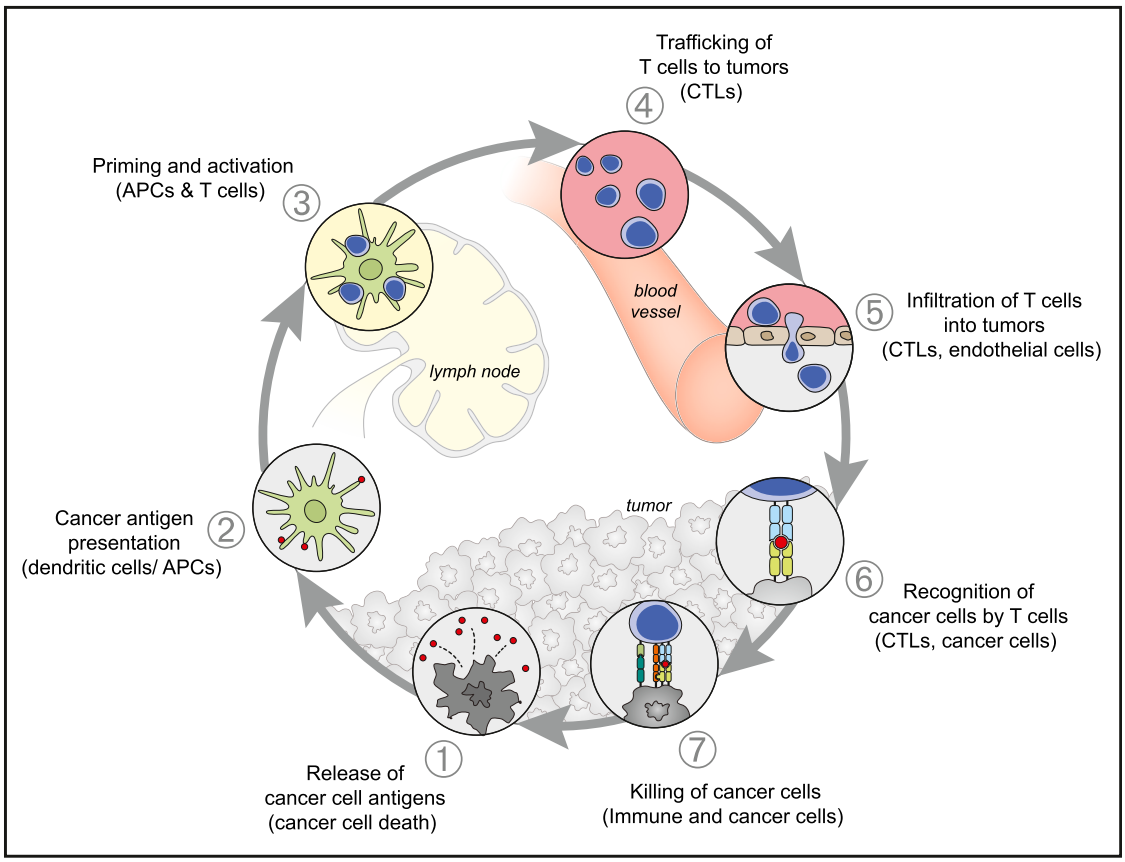

Figure 1. The Cancer-Immunity Cycle

The generation of immunity to cancer is a cyclic process that can be self propagating, leading to an accumulation of immune-stimulatory factors that in principle should amplify and broaden T cell responses. The cycle is also characterized by inhibitory factors that lead to immune regulatory feedback mechanisms, which can halt the development or limit the immunity. This cycle can be divided into seven major steps, starting with the release of antigens from the cancer cell and ending with the killing of cancer cells. Each step is described above, with the primary cell types involved and the anatomic location of the activity listed. Abbreviations are as follows: APCs, antigen presenting cells; CTLs, cytotoxic T lymphocytes.

to (step 4) and infiltrate the tumor bed (step 5), specifically recognize and bind to cancer cells through the interaction between its T cell receptor (TCR) and its cognate antigen bound to MHCI (step 6), and kill their target cancer cell (step 7). Killing of the cancer cell releases additional tumor-associated antigens (step 1 again) to increase the breadth and depth of the response in subsequent revolutions of the cycle. In cancer patients, the Cancer-Immunity Cycle does not perform optimally. Tumor antigens may not be detected, DCs and T cells may treat antigens as self rather than foreign thereby creating T regulatory cell responses rather than effector responses, T cells may not properly home to tumors, may be inhibited from infiltrating the tumor, or (most importantly) factors in the tumor microenvironment might suppress those effector cells that are produced (reviewed by Motz and Coukos, 2013).

The goal of cancer immunotherapy is to initiate or reinitiate a self-sustaining cycle of cancer immunity, enabling it to amplify and propagate, but not so much as to generate unrestrained autoimmune inflammatory responses. Cancer immunotherapies must therefore be carefully configured to overcome the negative feedback mechanisms. Although checkpoints and inhibitors are built into each step that oppose continued amplification and can dampen or arrest the antitumor immune response, the most effective approaches will involve selectively targeting the rate-limiting step in any given patient. Amplifying the entire cycle may provide anticancer activity but at the potential cost of unwanted damage to normal cells and tissues. Many recent clinical results suggest that a common rate-limiting step is the immunostat function, immunosuppression that occurs in the tumor microenvironment (Predina et al., 2013; Wang et al., 2013).

Initiating Anticancer Immunity: Antigen Release, Presentation, and T Cell Priming

Attempts to activate or introduce cancer antigen-specific T cells, as well as stimulate the proliferation of these cells over the last 20 years, have led to mostly no, minimal or modest appreciable anticancer immune responses. The majority of these efforts involved the use of therapeutic vaccines because vaccines can be easy to deploy and have historically represented an approach that has brought enormous medical benefit (reviewed by Palucka and Banchereau, 2013). Yet, cancer vaccines were limited on two accounts. First, until recently, there was a general lack of understanding of how to immunize human patients to achieve potent cytotoxic T cell responses. This limitation reflects continued uncertainties concerning the identities of antigens to

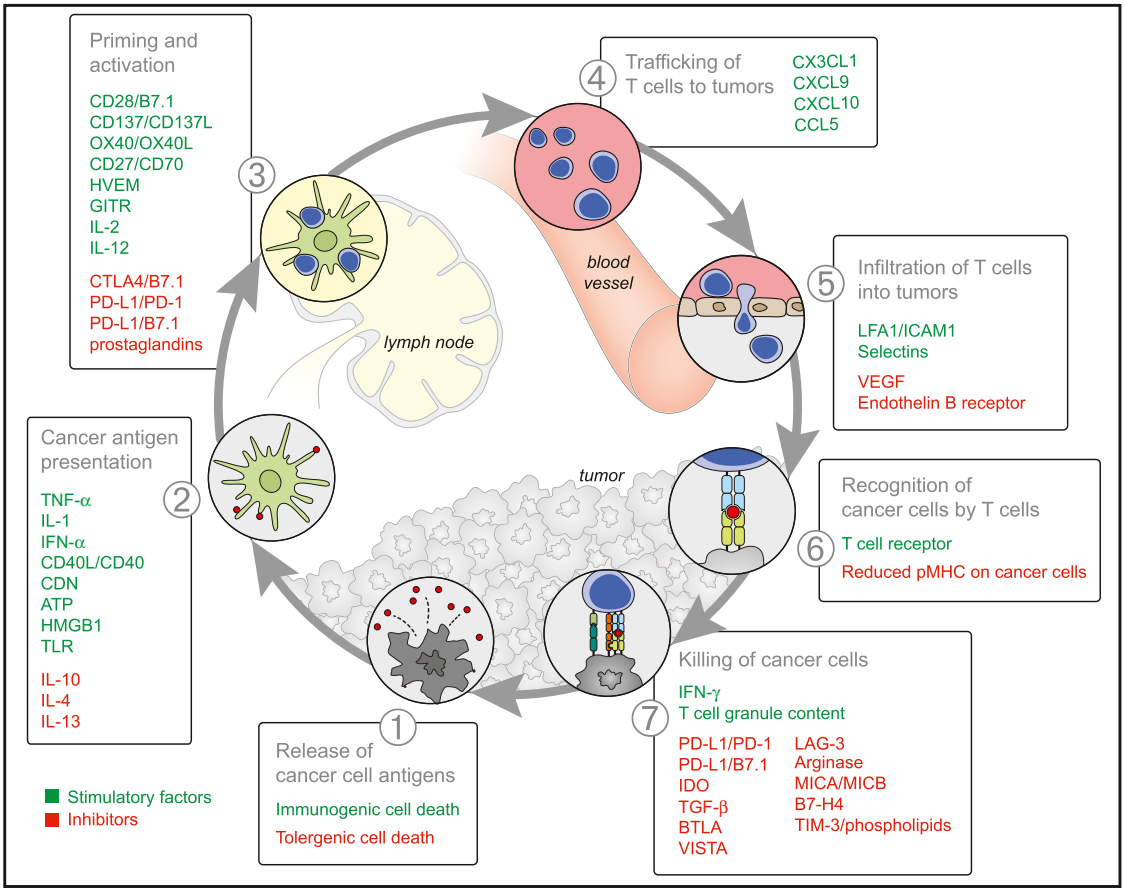

Figure 2. Stimulatory and Inhibitory Factors in the Cancer-Immunity Cycle

Each step of the Cancer-Immunity Cycle requires the coordination of numerous factors, both stimulatory and inhibitory in nature. Stimulatory factors shown in green promote immunity, whereas inhibitors shown in red help keep the process in check and reduce immune activity and/or prevent autoimmunity. Immune checkpoint proteins, such as CTLA4, can inhibit the development of an active immune response by acting primarily at the level of T cell development and proliferation (step 3). We distinguish these from immune rheostat ("immunostat") factors, such as PD-L1, can have an inhibitory function that primarily acts to modulate active immune responses in the tumor bed (step 7). Examples of such factors and the primary steps at which they can act are shown. Abbreviations are as follows: IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; CDN, cyclic dinucleotide; ATP, adenosine triphosphate; HMGB1, high-mobility group protein B1; TLR, Toll-like receptor; HVEM, herpes virus entry mediator; GITR, glucocorticoid-induced TNFR family-related gene; CTLA4, cytotoxic T-lympocyte antigen-4; PD-L1, programmed death-ligand 1; CXCL/CCL, chemokine motif ligands; LFA1, lymphocyte function-associated antigen-1; ICAM1, intracellular adhesion molecule 1; VEGF, vascular endothelial growth factor; IDO, indoleamine 2,3-dioxygenase; TGF, transforming growth factor; BTLA, B- and T-lymphocyte attenuator; VISTA, V-domain Ig suppressor of T cell activation; LAG-3, lymphocyte-activation gene 3 protein; MIC, MHC class I polypeptide-related sequence protein; TIM-3, T cell immunoglobulin domain and mucin domain-3. Although not illustrated, it is important to note that intratumoral T regulatory cells, macrophages, and myeloid-derived suppressor cells are key sources of many of these inhibitory factors. See text and Table 1 for details.

use, their mode of delivery, the types of adjuvants required, and the proximal characteristics of the desired T cell response (Palucka and Banchereau, 2013). Second, the presence of the immunostat in the tumor microenvironment may dampen or disable antitumor immune responses before clinically relevant tumor kill can occur. Thus, as long as these negative signals are in place, the prospects for vaccine-based approaches used alone are likely to be limited.

Although vaccination can accelerate the anticancer immunity in the context of treatments that suppress negative regulators (Palucka and Banchereau, 2013), a number of significant challenges need to be overcome. First is the identification of the appropriate tumor antigens to include in any vaccine. A large, monovalent antigen trial (using the C-T antigen MAGE-A3) is currently under way (Kruit et al., 2013; Vansteenkiste et al., 2013), yet it is not clear that any one candidate will necessarily generate sufficiently robust T cell responses in all patients. Moreover, a single antigenic target, especially one not derived from a protein that is an inherent oncogenic driver, seems more likely to enable resistance by antigenic drift (immune editing) than a multivalent vaccine (Palucka and Banchereau, 2013).

Deciding how to configure multivalent vaccines is itself a daunting challenge. It may be insufficient to rely entirely on sequencing the expressed tumor genome looking for point mutations, translocation fusions, or C-T antigens. Not only might this vary from patient to patient or even from cell to cell within a single patient's tumor, expression at the messenger RNA or protein level does not assure that predicted antigenic peptides will be generated and expressed as peptide-MHCI complexes, especially in the face of the allelic complexity in the MHC. Several groups are actively approaching this problem by using a combination of informatics and mass spectroscopy of peptides eluted from MHCI molecules

Table 1. Cancer-Immunity Cycle: Examples of Positive and Negative Regulators at Each Step

Steps	(+) Stimulators	(−) Inhibitors	Other Considerations	Example References
1. Release of cancer antigens	Immunogenic or necrotic cell death	Tolergenic or apoptotic cell death	Tumor-associated neoantigens and cancer testis antigens	Ferguson et al., 2011
2. Cancer antigen presentation	● Proinflammatory cytokines (e.g., TNF-α, IL1, IFN-α) ● Immune cell factors: CD40L/CD40 ● Endogenous adjuvants released from dying tumors: CDN (STING ligand), ATP, HMGB1 ● Gut microbiome products: TLR ligands	IL-10, IL-4, IL-13	Dendritic cell maturity	Lippitz, 2013; Mellman et al., 2011
3. Priming and activation	CD28:B7.1, CD137 (4-1BB)/CD137L, OX40:OX40L, CD27:CD70, HVEM, GITR, IL-2, IL-12	CTLA4:B7.1, PD-L1:PD-1, PD-L1:B7.1, prostaglandins	Central tolerance, T cell repertoire, T regulatory cells	Franciszkiewicz et al., 2012; Lippitz, 2013; Riella et al., 2012; So et al., 2006
4. Trafficking of T cells to tumors	CX3CL1, CXCL9, CXCL10, CCL5			Franciszkiewicz et al., 2012; Peng et al., 2012
5. Infiltration of T cells into tumors	LFA1:ICAM1, selectins	VEGF, endothelin B receptor		Franciszkiewicz et al., 2013
6. Recognition of cancer cells by T cells	T cell receptor	Reduced peptide-MHC expression on cancer cells		Mellman et al., 2011
7. Killing of cancer cells	IFN-γ, T cell granule content	PD-L1:PD-1, PD-L1:B7.1, TIM-3:phospholipids, BTLA, VISTA, LAG-3, IDO, Arginase, MICA:MICB, B7-H4, TGFβ	T regulatory cells, myeloid-derived suppressor cells, M2 macrophages, hypoxia	Chen et al., 2012; Greaves and Gribben, 2013; Mellman et al., 2011; Topalian et al., 2012a

on both cell lines and primary tumors (Kasuga, 2013; Rammensee et al., 2002; Segal et al., 2008). In principle, this information can be used to guide the formulation of multivalent vaccines, although it does not necessarily address the problem of how to identify MHC class II epitopes that may be required to provide CD4 T cell help that might be needed to produce protective CD8 responses. The use of intact proteins as an immunogen may help mitigate this issue. Moreover, it has thus far proved difficult to identify MHCI-bound peptides that harbor point mutations that could selectively target T cell responses to cancer cells, which is unfortunate given that targeting somatic mutations should reduce the chances of generating autoimmunity or the need to overcome central tolerance (Mellman et al., 2011). Even assuming the correct antigens are in hand, how best to deliver them to patients remains a critical unknown. Peptides in emulsified vehicles represent a common and accessible approach, but in the absence of compelling positive controls for any vaccine, it is impossible to know whether it is an effective approach. Other strategies include direct targeting to DCs, adoptive transfer of antigen-loaded DCs or tumor cells, recombinant viral vectors, and bacterial vectors (especially *Listeria*; reviewed in Kalos and June, 2013; Palucka and Banchereau, 2013). Work must continue evaluating each of these looking for pharmacodynamic read-outs of CD8 T cell responses. With the clinical success of anti-PD-L1 and anti-PD-1 antibodies, it should now be possible to evaluate different vaccines, adjuvants, and delivery approaches in combination and therefore under conditions that should enhance the ability to judge their relative efficacies with common clinical read-outs, such as partial or complete responses in established tumors.

Work on vaccines should continue in a systematic fashion with human studies, because animal models are unlikely to validate the best path forward (Davis, 2012). It is also unlikely that the best vaccine approaches will differentiate themselves any time soon, given the lack of direct comparisons in clinical studies. This represents a substantial logistical challenge to incorporating vaccination as part of a drug development plan. Not only are such trials long and expensive, but they also represent only one of many potential combinations that are competing to be evaluated in conjunction with other immunotherapies (Vanneman and Dranoff, 2012).

Therapeutic vaccination is not the only approach to accelerating and expanding the production of T cell immunity. Because anticancer T cells can be produced spontaneously, there is a growing appreciation that the tumor itself represents a type of endogenous vaccine. Accessing the naturally occurring source of cancer-associated antigens avoids problems associated with selection and delivery (Heo et al., 2013; van den Boorn and Hartmann, 2013). This approach is also convenient, but achieving it requires detailed knowledge around whether standard of care chemotherapy or targeted therapies are compatible with immunotherapies. Some therapies are thought to cause tumor cell death in a fashion that promotes immunity (reviewed in Zitvogel et al., 2013). However, it is unclear whether this effect can be accurately predicted and will, in any event, require empirical study. Chemotherapy, radiation therapy, and targeted therapies must also be evaluated for their effects on the immune system. Although it is assumed that many might be antagonistic, there are some reports that others might promote T cell activity

Table 2. Inhibitors of PD-L1 or PD-1 Currently Being Developed in Oncology

Therapeutic	Lead Company	Antibody Type	Affinity/K_d	Interaction Inhibited	Development
Anti-PD-L1					
MPDL3280A Herbst et al., 2013.	Genentech/Roche	Engineered IgG1 (no ADCC)	0.4 nM	PD-L1:PD-1 PD-L1:B7.1	Broad (lung pivotal)
MEDI-4736 Stewart et al., 2011.	AstraZeneca	Modified IgG1 (no ADCC)	Not available	PD-L1:PD-1 PD-L1:B7.1	Phase I
Anti-PD-1					
Nivolumab Brahmer et al., 2010.	Bristol-Myers Squibb	IgG4	2.6 nM	PD-L1:PD-1 PD-L2:PD-1	Broad (lung, melanoma, RCC pivotal)
Lambrolizumab Patnaik et al., 2012.	Merck & Co	IgG4 (humanized)	29 pM	PD-L1:PD-1 PD-L2:PD-1	Broad (melanoma pivotal)
Pidilizumab Rotem-Yehudar et al., 2009; Westin et al., 2012.	CureTech	IgG1 (humanized)	Not available		Broad
AMP-224 Smothers et al., 2013.	GlaxoSmithKline	PD-L2 IgG1 Fc fusion	Not available	PD-L1:PD-1 PD-L2:PD-1	Phase I

(Demaria et al., 2005; Duraiswamy et al., 2013; Hiniker et al., 2012; Ott et al., 2013; Postow et al., 2012; Stagg et al., 2011; Zitvogel et al., 2013).

Bypassing Vaccination by Adoptive T Cell Therapy

Another exciting development is that the initial demonstrations that genetically modified autologous T cells could be reinfused into patients to yield substantial clinical benefit, at least in certain B cell malignancies (Grupp et al., 2013; reviewed in Kalos and June, 2013). The most well developed of these is the use of "CARs," or chimeric antigen receptors, in which a patient's T cells are transfected with a construct encoding an antibody against a tumor surface antigen (typically CD19) fused to T cell signaling domains (Kochenderfer and Rosenberg, 2013). Similar approaches are under investigation with recombinant T cell receptors (reviewed in Kalos and June, 2013). The procedure avoids the need for immunization and may even overcome mechanisms of immune suppression by overwhelming the system through infusion of large quantities of the modified T cells. This can force the revolution of the Cancer-Immunity Cycle, enhancing the accumulation of stimulatory immune factors, and potentially promotes eventual self-propagation of the cycle. The potential limitations here, which are yet to be fully determined, include whether the approach can be extended to cancers beyond hematologic malignancies, whether the delivery of large numbers of monospecific T cells will cause resistance due to antigenic drift, and whether the toxicity issues already identified can be safely managed.

T Cell Priming and Activation

Whether tumor antigens are delivered exogenously or are captured and presented by DCs endogenously, another strategy for intervening in the Cancer-Immunity Cycle involves the control of T cell activation. This is the presumed primary mechanism of action of anti-CTLA4 antibodies, such as ipilimumab, which blocks the interaction of the major negative regulator of T cells (CTLA4) with its ligands B7.1 and B7.2 (CD80 and CD86; Qureshi et al., 2011). Thus, during antigen presentation in lymphoid organs (or in the periphery), the expansion of T cell responses is disinhibited, thereby promoting the production of autoreactive T cells, including tumor-specific T cells. The lack of selectivity in T cell expansion combined with the fundamental importance of CTLA4 as a checkpoint may underlie the significant immune-related toxicities seen in patients treated with ipilimumab (Hodi et al., 2010).

Nevertheless, the ability of this "lever" to create durable clinical responses in some patients has triggered a great deal of effort to seek other immune modulators; modulators that can achieve what ipilimumab can, but in a more selective and controllable fashion that will provide the potential for greater efficacy and frequency of response, with less autoimmune-related toxicity. In addition, the combination of ipilimumab with agents that modulate complimentary steps on the Cancer-Immunity Cycle are already underway (Karan and Van Veldhuizen, 2012; Madan et al., 2012), and preliminary results from combinations that inhibit tumor immunosuppression appear very promising in enhancing both antitumor immune responses and autoimmune toxicity (see below).

Immunostat Blockade: PD-L1 and PD-1

The identification of PD-L1 as a distal immune modulator expressed in 20%–50% of human cancer (Herbst et al., 2013) has led to the development of a number of cancer immunotherapies that target the interactions between PD-L1:PD-1, PD-L1:B7.1, and PD-L2:PD-1 (Table 2; reviewed in Chen et al., 2012; Topalian et al., 2012a). Anti-PD-L1 and anti-PD-1 monotherapy response rates have been presented for over 750 patients (ranging from 13% to 38%) treated across a broad range of human cancer types. Agents tested as monotherapy include MPDL3280A (anti-PDL1; Genentech/Roche; Cho et al., 2013; Hamid et al., 2013b; Herbst et al., 2013; Powderly et al., 2013; Spigel et al., 2013; Tabernero et al., 2013), nivolumab (anti-PD-1; Bristol Myers Squibb; Brahmer et al., 2013; Drake et al., 2013; Sznol et al., 2013; Topalian et al., 2013), and lambrolizumab (anti-PD-1; Merck; Hamid et al., 2013a). Antitumor activity of the PD-L1- and PD-1-targeted therapies that utilize an engineered immunoglobulin G1 (IgG1) (MPDL3280A; modified to eliminate ADCC by altering FcγR binding; Herbst et al., 2013) or IgG4 antibody (nivolumab and lambrolizumab; expected to reduce ADCC; Isaacs et al., 1996) backbone have resulted in particularly rapid antitumor activity, with some responses

observed within days of starting treatment. These data suggest that, for many human cancers, the Cancer-Immunity Cycle is intact up to the point of tumor cell killing by T cells, which can be potently restrained by PD-L1. Once the PD-L1:PD-1 interaction is blocked, preexisting anticancer T cells can have their effector function rapidly restored. This is consistent with the proposed mechanism of action of this negative regulator, where PD-L1 (expressed either on tumor cells or on tumor-infiltrating immune cells) binding to PD-1 on activated effector T cells causes the recruitment of the phosphatase SHP-2 and subsequent inactivation of the PI3 kinase-signaling cascade (Chemnitz et al., 2004; Parry et al., 2005). These events block the secretion or production of cytotoxic mediators required for killing. However, this block appears to be rapidly reversible once the inhibition is lifted.

Most importantly, the PD-L1 and PD-1 antagonists have exhibited significant response rates, and largely unprecedented durable responses. In melanoma, the anti-PD-1 antibody nivolumab has reported an overall response rate (ORR) of 31% (33/107) and a duration of response of 18.4 to 117.0+ weeks (Sznol et al., 2013), whereas lambrolizumab reported an ORR of 38% and duration of response of 1.9 to 10.8 months (Hamid et al., 2013a). Across a broad range of human cancers, which included lung, colon, head and neck, and gastric cancers in addition to melanoma and renal cell carcinoma, the anti-PD-L1 antibody MPDL3280A had an ORR of 21% (29% in melanoma, 22% in lung cancer) with 26 of 29 responses ongoing at the time of the report (time from starting treatment ranged from 3 to 15+ months) (Herbst et al., 2013; Hamid et al., 2013b; Spigel et al., 2013). Additionally, the safety profile of these agents suggests that while many cancer types express PD-L1 to inhibit anticancer immune responses, most patients do not have underlying autoimmunity inhibited only by PD-L1 expression (Francisco et al., 2010). Grade 3-4 treatment-related adverse events were noted to occur in 13% to 21% of patients treated (Hamid et al., 2013a; Herbst et al., 2013; Sznol et al., 2013). The majority of reported cases have been readily manageable with supportive care or by immune suppression with steroid administration. This is in stark contrast to the safety profiles of most therapies that target more proximal steps in the Cancer-Immunity Cycle (e.g., anti-CTLA4; Hodi et al., 2010) and might hint at the benefits of specifically targeting the properties of cancer that inhibit the immune response rather than nonspecific activation of the immune system. Although it is still relatively early in the development of these inhibitors (phase II/III trials are underway), the fact that three different antibodies have yielded such results greatly increases the confidence in a positive outcome.

From a drug development and clinical care perspective, the activity observed with anti-PD-L1 or anti-PD-1 is clear. Robust single-agent activity was observed rapidly and for extended durations without identified off-target toxicity (Topalian et al., 2013). This situation is distinct from the majority of other agents under investigation (or approved) in oncology, except for a select group of small-molecule inhibitors that target driver oncogenic translocations or mutations (e.g., imatinib for *BCR-Abl* [Lin et al., 2013], crizotinib for *ALK* translocations [Rothschild and Gautschi, 2013], vemurafenib [Huang et al., 2013], and dabrafenib [Huang et al., 2013] for the V600E *BRAF* mutation and erlotinib for mutant EGF receptor [Bulgaru et al., 2003]). Therefore, extended trials looking for incremental effects or complex combination approaches should not be necessary. Furthermore, the potential for biomarker-driven patient selection to optimize treatment benefit appears promising and might distinguish patients most likely to have strong benefit from the inhibition of PD-L1:PD-1 as monotherapy opposed to those that may most likely require a different or combinatorial approach (Powderly et al., 2013; Topalian et al., 2013). These results also emphasize the likely importance of immunosuppression in the natural history of cancer. Unfortunately, as the clinical trial data to date confirm, the majority of patients will not respond or will respond only incompletely to PD-L1 or PD-1 inhibitors. Because multiple other mechanisms of immunosuppression are known that may work together or in parallel with PD-L1:PD-1-mediated inhibition, there is a need to pursue other potential agents that exhibit the same profile of rapid, substantial responses. For example, many tumors are characterized by significant infiltration by T regulatory cells, and targeting these may prove to be a fruitful approach (Jacobs et al., 2012). It is possible that even ipilimumab works, at least in part, by causing T regulatory cell (Treg) depletion.

Combination Immune Therapies

It is reasonable to suspect that immunotherapy approaches, from vaccines to CARs, would be more effective when given in combination with a PD-L1 or PD-1 inhibitor (Goding et al., 2013; West et al., 2013). By disabling the immune inhibition in the tumor microenvironment, approaches that target earlier steps in the Cancer-Immunity Cycle (steps 1–6) are more likely to lead to cancer cell killing. Conversely, PD-L1 or PD-1 inhibition may not be sufficient for optimal antitumor activity in some patients, particularly those that do not demonstrate evidence of tumor immune cell infiltration (Gajewski et al., 2011, 2013; Gajewski, 2012). PD-L1- and PD-1-targeted therapies suggest that in patients whose tumors express PD-L1, the proximal steps of the Cancer-Immunity Cycle are intact and may not require further enhancement. These patients are most commonly the patients who exhibit rapid and durable response to PD-L1 or PD-1 inhibition. However, although some PD-L1-negative tumors still respond to PD-L1 or PD-1 monotherapy, the majority of tumors do not (Powderly et al., 2013; Grosso et al., 2013). This outcome can be indicative of patients who have a defect in steps 1 to 6 of the Cancer-Immunity Cycle and may be most commonly a defect in cancer antigen-specific T cell activation or infiltration of T cells into tumors (Powderly et al., 2013). However, more data from human tumors are likely to be necessary to further elucidate what critical breaks in the cycle are most prominent in different human cancers.

One approach, combining a CTLA4 targeted therapy (ipilimumab) with a PD-1-targeted inhibitor (nivolumab), appears to enhance the immune activity in patients over either therapy alone in an early phase study (Wolchok et al., 2013). Anti-CTLA4 can lead to enhanced priming and activation of antigen-specific T cells and potentially clearance of regulatory T cells from the tumor microenvironment (Table 1). The blocking of PD-L1 or PD-1 can remove the inhibition of cancer cell killing by T cells (Figure 3). By inhibiting two targets that affect two steps in the Cancer-Immunity Cycle, rapid and deep responses (by modified WHO criteria) were observed in patients with melanoma (ORR: 40% [21/52]; CR: 10% [5/52]). Immune-related toxicities were also enhanced in their magnitude, frequency, and onset (53%

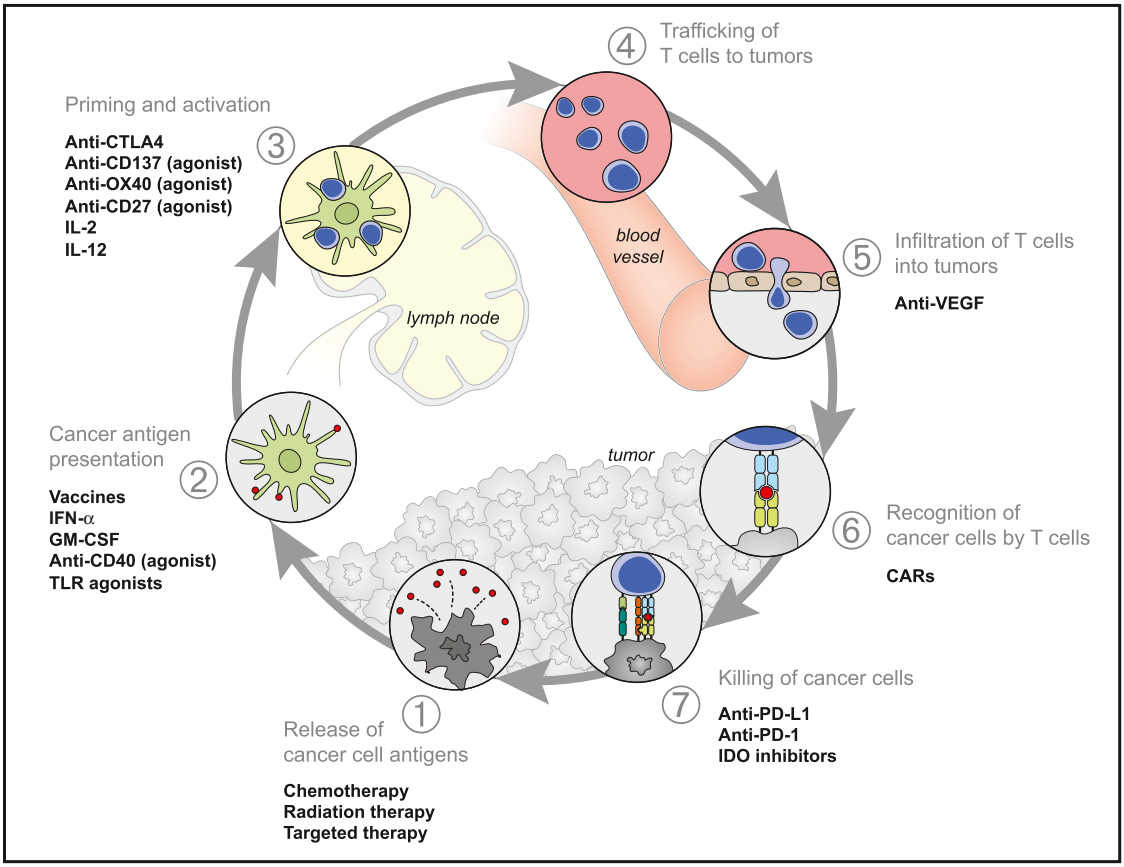

Figure 3. Therapies that Might Affect the Cancer-Immunity Cycle

The numerous factors that come into play in the Cancer-Immunity Cycle provide a wide range of potential therapeutic targets. This figure highlights examples of some of the therapies currently under preclinical or clinical evaluation. Key highlights include that vaccines can primarily promote cycle step 2, anti-CTLA4 can primarily promote cycle step 3, and anti-PD-L1 or anti-PD-1 antibodies can primarily promote cycle step 7. Although not developed as immunotherapies, chemotherapy, radiation therapy, and targeted therapies can primarily promote cycle step 1, and inhibitors of VEGF can potentially promote T cell infiltration into tumors—cycle step 5. Abbreviations are as follows: GM-CSF, granulocyte macrophage colony-stimulating factor; CARs, chimeric antigen receptors.

Grade 3-4 treatment-related toxicities). Although many of these were serious and required treatment, therapy discontinuation, or hospitalization, the durable partial and complete responses in melanoma may warrant this approach in some patients. In particular, combination therapy appeared to most dramatically benefit patients who were less likely to benefit from PD-L1 or PD-1 inhibition alone, because their tumors were PD-L1-negative (6/13 PD-L1-positive and 9/22 PD-L1-negative patients responded to combination therapy; Wolchok et al., 2013). The addition of a CTLA4-targeted therapy may be completing the defect in the Cancer-Immunity Cycle for patients who are PD-L1-negative. Further studies of preipilimumab and on ipilimumab treatment tumor samples are warranted to better understand this effect.

Other combinations that merit serious consideration include anti-PD-L1 or anti-PD-1 with vaccination, especially if it becomes possible to monitor a patient's T cell profile to distinguish individuals who have generated suboptimal T cell responses to their cancers (Duraiswamy et al., 2013; Ge et al., 2013). In addition, combinations with agents that will enhance T cell trafficking and infiltration into the tumor bed should be explored vigorously, because the entry step may be important in some patients. In this class, inhibition of VEGF by the anti-VEGF antibody bevacizumab appears to be a promising candidate based on hints from the preclinical and clinical literature (Motz and Coukos, 2013; Hodi et al., 2010). Similarly, B-Raf inhibitors (vemurafenib) may also enhance T cell infiltration into tumors (Liu et al., 2013). Of course, with increased activity due to combinations comes the increased chance for additive or synergistic toxicity. This further highlights the importance of selecting therapeutic targets that are specific to the ability of a tumor to escape immune eradication over targets that may also play an important role in mediating immune homeostasis and preventing autoimmunity.

Concluding Remarks

The objective of understanding the inherent immune biology related to cancer is to better define strategies to harness the human immune response against cancer to achieve durable responses and/or complete eradication of cancer in patients safely. Multiple approaches to cancer therapy exist, and few are as complicated as immune-based therapy. Multiple numbers of systemic factors can effect or contribute to the success or failure of immune therapy and lends to this complexity. Results may be confounded by many currently unmeasured variables,

including any given patient's gut microbiome, their diet, and whether they contract an underlying infection (Brestoff and Artis, 2013; James et al., 2012, Rothman and Paterson, 2013; Xu et al., 2012). Yet, as complicated and incompletely understood human immunology may be, the immune response to cancer may be less complicated and less protean than the biology of cancer cells themselves.

The early data from clinical studies of immune-based therapies suggest durable activity that few cancer therapies can approximate. The immune response can be rapid, durable, and adaptable. Once activated, it has the potential to be self-propagating (for example, see Hamid et al., 2013a). These characteristics may preempt the development of secondary resistance seen with most cancer therapies. In fact, with each revolution of the cycle, not only can an accumulation of immune stimulatory factors occur (Powderly et al., 2013), reinforcing the ongoing antitumor immune response, but it can also stimulate the generation of new antitumor immune responses by promoting antigen spreading (for example, see Corbière et al., 2011). As much as immune responses can completely and safely eradicate viral infections, the goal of cancer immunotherapy should remain the complete and safe eradication of cancer from individual patients. Meeting this objective may require only monotherapy approaches in some patients, whereas others may require combined therapies. By understanding the biology present in specific patients, immune-related biomarkers may allow us to map out the Cancer-Immunity Cycle for individual patients and enable tailoring of specific immune therapies or combinations of immune therapies.

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