The Basis of Oncoimmunology

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Cancer heterogeneity, a hallmark enabling clonal survival and therapy resistance, is shaped by active immune responses. Antigen-specific T cells can control cancer, as revealed clinically by immunotherapeutics such as adoptive T-cell transfer and checkpoint blockade. The host immune system is thus a powerful tool that, if better harnessed, could significantly enhance the efficacy of cytotoxic therapy and improve outcomes for cancer sufferers. To realize this vision, however, a number of research frontiers must be tackled. These include developing strategies for neutralizing tumor-promoting inflammation, broadening T-cell repertoires (via vaccination), and elucidating the mechanisms by which immune cells organize tumor microenvironments to regulate T-cell activity. Such efforts will pave the way for identifying new targets for combination therapies that overcome resistance to current treatments and promote long-term cancer control.

Introduction

Cancer is an insidious disease traditionally classified by cell and tissue type of origin. Cancer has historically been treated according to a "one size fits all" approach based on broad pathologic criteria and involving various regimens of cytotoxic therapy. With the advent of modern sequencing methodologies, however, we now appreciate that significant genomic, transcriptomic, and epigenetic heterogeneity exists within individual tumor types; this recognition has enabled subclassification of tumors of common origin. This, in turn, has led to improved outcomes for some cancer types, as response rates to targeted and cytotoxic therapies increase when patients are stratified based on the molecular characteristics of their tumors. Examples include imatinib in chronic myelogenous leukemia (Druker et al., 2006), HER2-targeted therapies for HER2-positive breast cancer (Shepard et al., 1991), and estrogen antagonists for estrogen-receptor-positive breast cancers (Heiser et al., 2012). These molecular advances helped to usher in a new era of precision medicine that is reshaping clinical treatment across the cancer spectrum. However, there remain significant fractions of patients that do not respond to "designer" therapies even when their tumors are classified based on molecular and pathologic criteria. Additional tumor or systemic characteristic(s) are thus unaccounted for that not only impact neoplastic growth and dissemination, but also impact response to therapy.

Recent seminal in vivo studies revealed that neoplastic cells rely on the diversity of normal resident and recruited accessory cells to support their evolution (Hanahan and Coussens, 2012). Accessory cells are now recognized as "neoplastic cell-extrinsic hallmarks of cancer" and include those forming the hematogenous and lymphatic vasculature, tissue-specific mesenchymal support cells, and myeloid and lymphoid-lineage immune cells. Accessory cells integrate with the dynamic soluble and insoluble matrices constituting the "tumor stroma"; collectively, they fuel neoplastic evolution (Hanahan and Coussens, 2012). In other words, reciprocal interactions between accessory cells, their mediators, structural components of the extracellular matrix (ECM), and genetically altered neoplastic cells regulate all aspects of tumorigenicity. These realizations fueled the development of anti-cancer agents targeting the vasculature (Kerbel, 2011) and, more recently, propelled clinical investigations into the efficacy of immune therapeutic approaches that neutralize tumor-promoting chronic inflammation and/or embolden or unleash cytotoxic activities of antigen-specific T cells (Coussens et al., 2013; Pardoll, 2012).

Indeed, cancer is visible to the immune system, i.e., immunogenic, during early neoplasia. Classic studies from Schreiber and colleagues in mice with carcinogen-initiated sarcomas revealed that the immune system could recognize and reject cancerous cells (Dunn et al., 2004). Elimination can be explained by cytotoxic antigen-specific T cells responding to relatively high mutational burdens induced by carcinogens and thus providing neo-antigens for T-cell priming; these findings established the principles of elimination, equilibrium, and eventually escape when neoplastic cells become invisible to the immune system (Dunn et al., 2004). Neoplastic cells in part escape when tumor arises out of chronically inflamed tissues—there, chronic infiltration of tissue by leukocytes (e.g., type 2 cytokine-activated myeloid cells and immune-suppressive B, T, and myeloid subsets) subvert T-cell-directed elimination and thus aid tissue-based programs, e.g., angiogenesis, lymphangiogenesis, matrix remodeling, etc., supporting neoplastic progression (Coussens et al., 2013).

Mounting observations in humans support the concept that cancer initiation and progression are significantly impacted by altered or misled immune responses (Figure 1). Individuals suffering from chronic inflammatory conditions are at increased
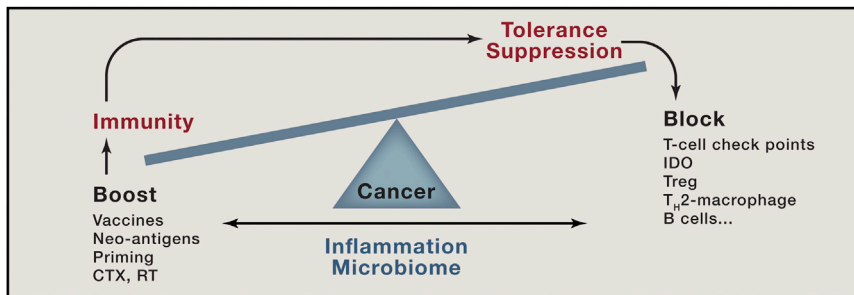
Figure 1. The Makings of Tumor Immunity

The communication between cancer and the immune system is a dynamic process, reminiscent of a balance. When immunity to cancer is "up" and the suppressive processes are "down," cancer is under control. However, a strong anti-tumor immune response will trigger largely physiological processes designed to dampen effector T cells to prevent tissue damage and maintain tissue homeostasis. Given that the immunity might have evolved mainly to maintain self, to establish coexistence with environment, and to occasionally protect self from external threats, the suppression prevails. Multiple pathways of suppression are at play in tumor microenvironments, including cells such as T_H2-polarized macrophages, immature and suppressive monocytes, regulatory B cells, and regulatory T cells, as well as molecules such as checkpoints that control T-cell differentiation (for example, CTLA-4 and IDO) and effector function (such as PD-1). Pharmacological blockade of these inhibitory pathways can tip the balance toward anti-cancer effector T cells. The latter ones can be primed or boosted by antigen-presenting cells (DCs) and/or by co-stimulatory signals (for example, CD137 ligands). Recent studies demonstrate that thymus-independent neo-antigens generated in adult life by somatic mutation or post-translational regulation (for example, phosphorylation) might be more immunogenic (or perhaps linked with less suppression) than shared tumor antigens. Neo-antigens can occur as random results of somatic mutation, as well as a by-product of anticancer treatments, e.g., chemotherapy (CTX) or radiation therapy (RT), or by targeting epigenetic control mechanisms or drugs intervening with DNA repair pathways. They can be presented to T cells in exogenous vaccines, as well as endogenously via DCs that captured dying neoplastic cells. When T cells specific to defined antigens kill neoplastic cells, such a process can enable generation of responses to other antigens, so called epitope spreading. A critical factor in the balance between immunogenicity and suppression is inflammation (which, in turn, is impacted by the microbiome); indeed, the type of inflammation (tumor-destructing T_H1 or tumor-promoting T_H2 and T_H17) should become a part of TNM grading, along with pathology, microbiome phenotype, and immune infiltrate assessment.

risk for developing cancer (Thun et al., 2004). Incidence of viral (DNA tumor virus) and carcinogen-associated cancers is increased in immune-compromised individuals, even as the relative risk of cancer types lacking viral or carcinogen etiology is diminished (reviewed in de Visser et al., 2006). Age-related immunosenescence likely plays a role in increased incidence of malignancy in aged individuals (Campisi et al., 2011). The advent of some biologic therapies impacting how tissues activate and resolve inflammation, e.g., tumor necrosis factor (TNF) blockade (Bongartz et al., 2006), also skews cancer incidence metrics. However, the role(s) that immune pathways play in driving malignancy remains to be clarified. How does the immune system recognize tissue-specific mediators triggering and maintaining chronic inflammatory responses? What oncogenic events and altered metabolic states lead to the generation of neo-antigens that in turn induce T-cell responses? What physiological mechanisms regulate immune homeostasis such that (acute) inflammation can be resolved as rapidly as it is activated (a critical control program to thwart autoimmunity)? What is the role of the host microbiota in regulating systemic immune responses to neoplasia? How do neoplastic cells survive immune attack by T cells? These questions are in need of answering to effectively move cancer research and cancer medicine forward.

A common feature of all cancers, regardless of origin, is prominent presence of diverse assemblages of immune cells (Coussens et al., 2013). The consequences of such infiltrates on the fate of cancerous cells are diverse (Figure 2). For example, under continual immune pressure, i.e., antigen presentation to T cells, neoplastic cells become "immune-edited" to escape immune surveillance (Dunn et al., 2004) and instead co-opt immune cells to favor their sustained proliferation (Balkwill et al., 2005). Nonetheless, recent studies demonstrate that the presence of lymphoid aggregates is linked with improved responses to cancer therapies—for example, standard cytotoxic therapies, vaccine-based treatments, or immune checkpoint blockade (Topalian et al., 2015). Such "hot" tumors are thus more amenable to control than "cold" tumors, i.e., tumors with diminished T-cell infiltrates, thus driving modern cancer medicine to investigate how to reprogram the tumor microenvironment (TME) to attract the right type of immune infiltrate. This topic, along with other open questions in the field of oncoimmunology, are discussed here.

The Makings of the Immune Response to Cancer

Tumors are organized tissues with numerous reciprocal local and systemic connections with immune cell populations of both the myeloid and lymphoid lineages. Here, we summarize the key myeloid and lymphoid populations regulating the immune response to cancer and how the fundamental physiological processes that they govern are harnessed for neoplastic progression and tumor formation.

The Myeloid Compartment

Myeloid cells have multiple homeostatic functions that are co-opted by evolving neoplasms; these can be roughly summarized as: (1) antigen capture for degradation (macrophages) or presentation (dendritic cells [DCs]); (2) tissue repair (macrophages), and (3) effector functions (mast cells, monocytes, and granulocytes). Neoplastic cells can alter the steady-state activity of all myeloid cells present in the TME, including tissue-resident and blood-derived cells, by secreting factors such as interleukin (IL)-6 or granulocyte-macrophage colony-stimulating factor (GM-CSF), that increase recruitment and proliferation of immature myeloid cells atypical under physiological conditions (Gabrilovich et al., 2012).

An important feature of myeloid cells is their functional plasticity in response to environmental signals. This property can dictate such opposite outcomes as antigen degradation or antigen presentation when macrophages acquire DC capabilities (Bancherreau et al., 2000), tissue repair rather than inflammation when macrophages are polarized toward type 2 states, and protective or non-protective T-cell immunity when programmed by cancer-derived factors (Balkwill et al., 2005). Thus, plasticity
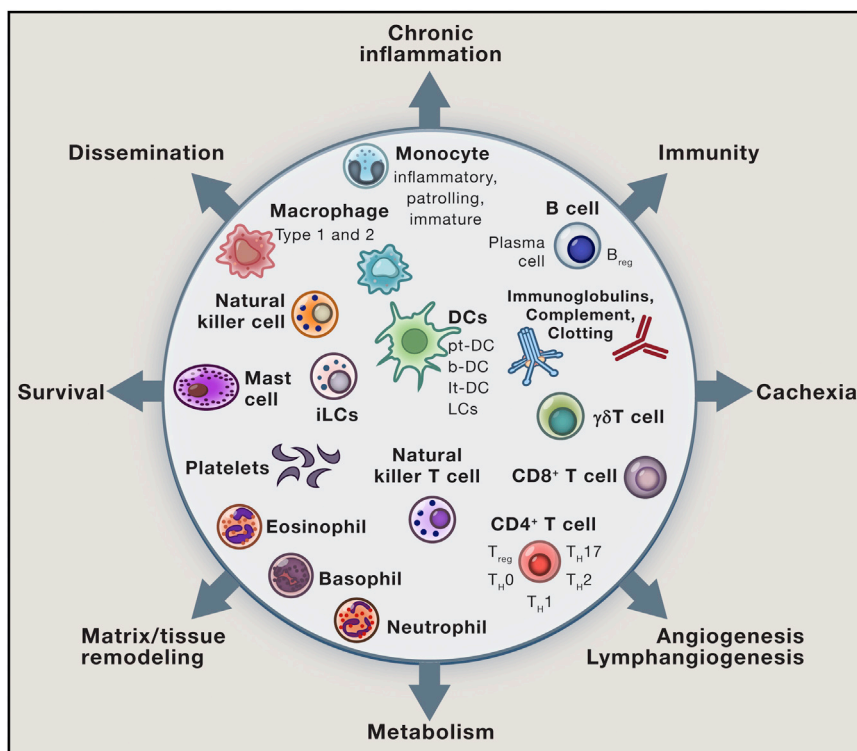
Figure 2. Immune-Mediated Landscape

The yin and yang implications of tumor-immune system communications form the basis for disease pathophysiology and, at the same time, targets for therapeutic intervention. The disease landscape emerging from these multi-factorial interactions is orchestrated by the three compartments, i.e., the cancer, the immune system, and the host. The outputs are numerous and dramatically opposite, as well as both local and systemic, and include: immunity that might control cancer; chronic inflammation that can be linked with tissue remodeling processes and metabolic changes that support neoplastic cell survival and primary tumor development; angiogenesis and lymphangiogenesis that can also support metastatic dissemination; as well as systemic consequences for the host including cachexia. Clearly, therapy going forward will require a well-timed and orchestrated combination of therapies, targeting multiple modes of communication and effect, to combat this multi-factorial disease, taking into account the patient's steady-state commensal bacteria complexity and load and how that is impacted by therapy.

and communication within the myeloid compartment and between myeloid and other immune cells and stromal components is critical for tumor formation.

Cancer Antigen Presentation and Dendritic Cells. Cancer antigens are presented to T cells either at tumor sites or in draining lymph nodes by DCs (Figure 3). Cancer antigens, soluble and cell borne, are transported to lymph nodes via lymphatic vessels. Soluble antigen is captured by lymph-node-resident DCs, while tissue-resident DCs capture antigen at tumor sites; either population can present antigen locally or migrate through lymphatic vessels to present in lymph nodes (Steinman, 2011). DCs display protein antigens in the context of classical major histocompatibility (MHC) class I and MHC class II molecules or lipid antigens in the context of non-classical CD1 molecules that allow selection of rare antigen-specific T lymphocytes, including CD8+ T cells, CD4+ T cells, and NK T cells. Compared with other antigen-presenting cells (APCs), DCs are extremely efficient in their ability to induce antigen-specific T-cell responses, justifying their name "professional APCs" (Lanzavecchia and Sallusto, 2001). Naive CD8+ T cells differentiate into cytotoxic T lymphocytes (CTLs) in lymphoid organs upon encounter with DCs, presenting tumor-derived peptides in the context of co-stimulation through CD80, CD70, and 4-1BB, as well as through DC-derived cytokines such as IL-12, type I interferon, and IL-15 (Steinman, 2012). The priming of new T-cell repertoires during tumorigenesis may be critical for clinical success of therapeutic agents aiming to unleash antigen-specific CTL activities. Naive CD4+ T cells can give rise to helper cells with distinct cytokine profiles or to Fox-P3+ regulatory T cells (Treg), whose role is to dampen CTL activity and avoid autoimmune responses (Zhu and Paul, 2008).

DCs express numerous pattern recognition receptors, including lectins, Toll-like receptors (TLRs), NOD-like receptors (NLRs), and helicases, through which they can sense microbes and tissue damage (cancer) such as increased pericellular nucleic acids (Pulendran, 2015). If DCs do not receive maturation signals, such as when exposed to high levels of IL-10 (Ruffell et al., 2014), they remain immature and antigen presentation instead leads to T-cell suppression. DC plasticity in response to extrinsic signals, together with the existence of discrete subsets with unique functions, empowers DCs as key initiators and regulators of the immune response (Pulendran, 2015). We will illustrate this point briefly; mouse and human DC subset biology was recently reviewed elsewhere (Merad et al., 2013).

The diversity of human DC subsets was revealed by studies of blood and skin DCs. Three main cell-surface markers distinguished human-blood-circulating DC subsets: CD303 (BDCA-2) on plasmacytoid DCs (pDCs), CD1c (or BDCA-1) expressed on the majority of circulating DCs, and CD141/BDCA-3 expressed on a small fraction (Merad et al., 2013). Human CD141+CD1c− DCs uniquely express TLR3, produce IL-12, and efficiently cross-prime CD8+ T cells when activated with poly I:C (Joffre et al., 2012). However, other human DCs, such as epidermal Langerhans cells and CD1c+ DCs, also cross-present antigens to CD8+ T cells. Indeed, our studies have unraveled the basic principles by which human DC subsets differentially regulate CD8+ T cells (Klechevsky et al., 2008). Thus, human Langerhans cells are highly efficient at priming cytotoxic CD8+ T cells, while CD14+ dermal DCs prime type 2 cytokine-secreting CD8+ T cells (Klechevsky et al., 2008). Blood- and tissue-resident CD1c+ DCs, but not CD141+ DCs, exposed to live-attenuated influenza virus promote CD103 (αE integrin) expression on CD8+ T cells and their accumulation in epithelia (Yu et al., 2013).
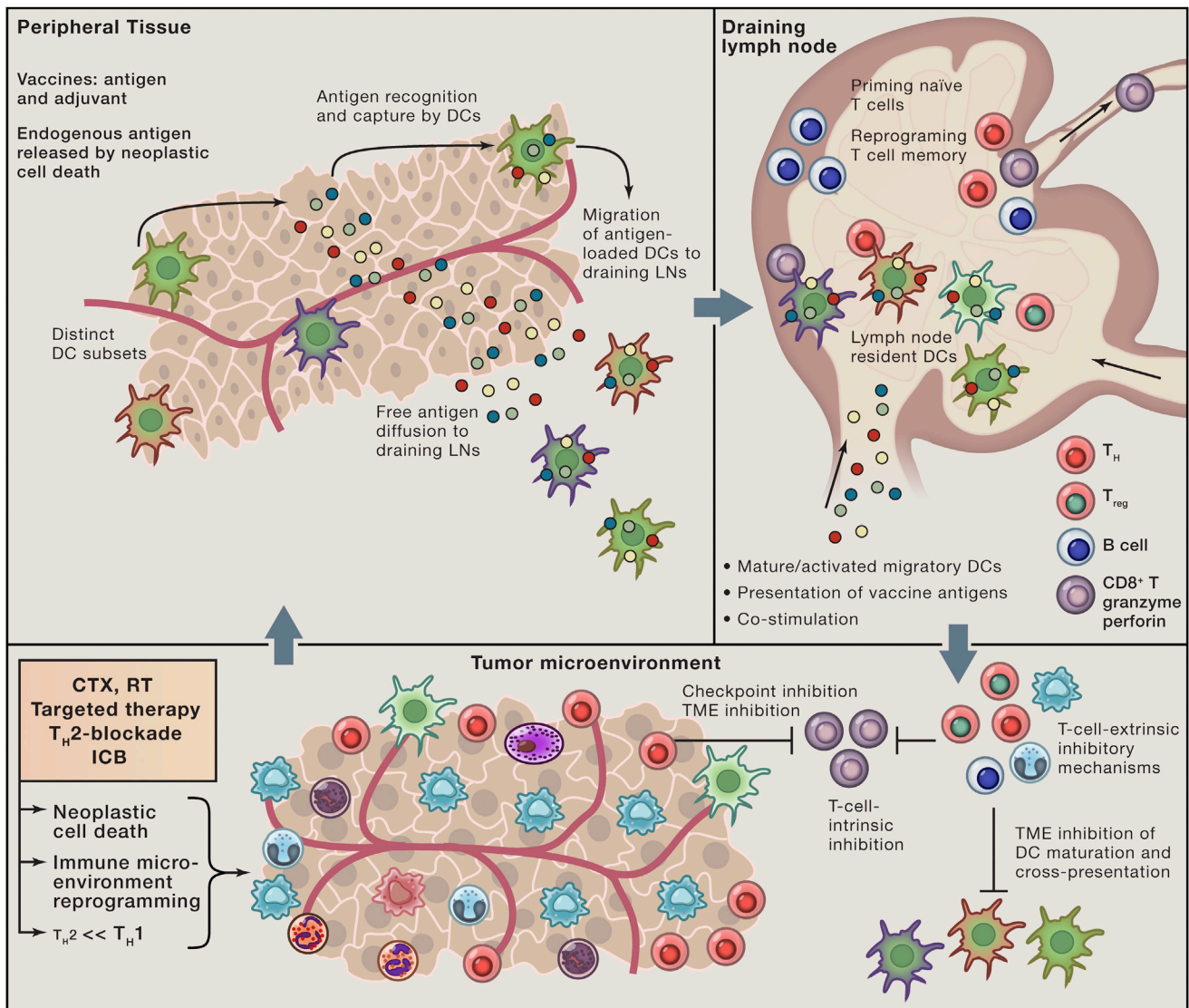
Figure 3. The Priming of Cancer Immunity

The cycle of anti-tumor immunity starts presumably with presentation of cancer antigens liberated in the process of cell turnover; this same pathway can be followed for vaccination, as illustrated herein. Antigens are sensed and captured either by tissue-resident DCs or by DCs in draining lymph nodes (LNs). DCs initiate an immune response by presenting captured antigens, in the form of peptide-major histocompatibility complex (MHC) molecule complexes, to naive (that is, antigen inexperienced) T cells in lymphoid tissues. When compared with other APCs, such as macrophages, DCs are extremely efficient and can elicit very low numbers of T cells to respond. Naive CD8+ T cells differentiate into CTLs in lymphoid organs upon encounter with DCs presenting tumor-derived peptides in the context of co-stimulation through CD8, CD70, and 4-1BB, as well as DC-derived cytokines such as IL-12 and IL-15. Naive CD4+ T cells can give rise to helper cells (e.g., T_H) with distinct cytokine profiles or to regulatory T cells (Treg) whose role is to dampen the immune response. T cells migrate through blood and lymphatics. Upon arrival in tumor beds, CD8+ T cells must confront numerous barriers including: (1) intrinsic regulators, for example, CD28-CTLA-4, PD1-PDL1, and ILTs, as well as extrinsic regulators cells such as Tregs, Bregs, or myeloid cells; (2) a corrupted TME with pro-tumor inflammation; (3) impaired cross-presentation due to TME-based DC inhibition; (4) antigen loss and immune evasion of tumor target; and (5) tissue-specific alterations such as fatty cells in breast cancer or desmofibrosis in pancreatic cancer stroma. Killing of tumor cells either via T cells or by standard therapy can lead to endogenous antigen release and DC activation, so called "endogenous vaccination," thereby closing the cycle. Inevitable to this is the induction of tissue resistance mechanisms, for example, expression of PD-L1 on neoplastic or other immune cells, as the result of powerful effector immunity, including actions of IFNγ. Thus, future immunotherapy approaches will be based on combinations of different therapeutics targeting distinct components of this cycle, for example, via intratumoral delivery of activating agents able to reprogram the function of infiltrating leukocytes.

The Lymphoid Compartment

The lymphoid compartment in tumors includes natural killer (NK) cells, γδ T cells, NK T cells, CD4+ T cells, CD8+ T cells, and B cells. Their functional activity depends upon expression of restriction elements, including peptide-MHC complexes (pMHC; for T cells), the MHC class I molecule (for NK cells), or surface proteins (for B-cell products, i.e., antibodies) that can be recognized in a specific manner. In addition, lymphoid cells can be induced to secrete different types of cytokines based on effector functions. For example, following an activating stimulus,

T$_H$1-polarized CD4$^+$ T cells secrete IL-2, TNFα, and IFNγ; in conjunction with cytotoxic CD8$^+$ T cells, they promote macrophage cytotoxic activity (Stout and Bottomly, 1989) and can induce upregulation of antigen processing and expression of MHCI and II molecules in professional APCs (i.e., macrophages and DCs). In contrast, expression of IL-4, -5, -6, -10, and -13 by T$_H$2-polarized CD4$^+$ T cells can induce T-cell anergy and loss of T-cell-mediated cytotoxicity, enhance humoral immunity, and regulate the tumor-promoting activities of macrophages (DeNardo et al., 2009).

CD8$^+$ T cells are considered the major anti-cancer effector cells, as they can give rise to CTLs that kill neoplastic cells presenting a specific pMHC complex (Appay et al., 2008). CTLs can be generated through either the priming of naive T cells or reprogramming of memory T cells. Naive CD8$^+$ T cells differentiate into CTLs in lymphoid organs upon encounter with APCs presenting tumor-derived peptides in the context of appropriate co-stimulation and cytokine help. The ideal properties of anti-cancer CD8$^+$ T cells include: high affinity for pMHC on tumor cells; high levels of cytotoxic mediators, e.g., granzymes A and B and perforin; expression of surface molecules, allowing trafficking into the tumor; and extended longevity and memory, thus enabling CTL generation upon antigen re-exposure (Appay et al., 2008).

Memory T cells have long been described as two circulating populations: (1) central memory T cells that migrate between the secondary lymphoid organs and are capable of mounting proliferative responses on pathogen re-encounter and (2) effector memory T cells that traffic between blood and extralymphoid compartments for peripheral immune surveillance (Mueller et al., 2012). Tissue-resident memory T cells are a third and phenotypically distinct category. Studies in mice and humans have revealed that this latter population can be superior to circulating central memory T cells at providing rapid long-term protection against re-infection (Sheridan and Lefrançois, 2011). Therefore, an active mechanism of peripheral T-cell retention likely exists not only to facilitate clearance of infected cells, but also to promote accumulation at sites having cleared an infectious virus. CD103/β7 integrin endows peripheral CD8$^+$ T cells with a unique capacity to access epithelial compartments. Expression of CD103 on CTLs mediates adherence to E-cadherin and appears to be important in the final stages of neoplastic cell lysis and rejection (Le Floc'h et al., 2007). Indeed, for mucosal cancer vaccines, homing to and retention of CD8$^+$ T cells in mucosa is critical for efficacy (Sandoval et al., 2013).

Upon arrival in tumor beds, CD8$^+$ T cells must confront numerous barriers, including intrinsic checkpoint regulators, such as CD28-CTLA-4, PD1-PD-L1, and immunoglobulin-like transcript receptors (ILTs) (Pardoll, 2012); extrinsic checkpoint regulators, such as Treg cells (Fehérvari and Sakaguchi, 2004) or myeloid cells (Gabrilovich et al., 2012); a corrupted TME with protumor inflammation (Coussens et al., 2013); antigen loss and immune evasion of tumor targets (Klebanoff et al., 2011); and tissue-specific alterations, such as fatty cells in breast cancer or desmofibrosis in pancreatic cancer stroma. Defining strategies for bypassing these obstacles and improving the clinical efficacy of T-cell therapies is the object of intense study.

An important concept recently proposed by Mellman and colleagues is the cancer-immunity cycle (Chen and Mellman, 2013). It becomes apparent that any effective immune response against cancer will generate resistance via physiological pathways that evolved to protect tissue homeostasis. Here, we discuss how this cycle is altered in cancer pathogenesis and how it can be harnessed therapeutically. Clearly, combination therapies that intervene at several distinct pathways within the cancer-immunity cycle are needed to achieve cancer control.

Chronic Inflammation and Alterations of Leukocyte Compartments in Cancer
Basic Principles

Unabated inflammation is a hallmark of cancer and is mediated by immune cells attracted to or residing at sites of neoplastic transformation (Balkwill et al., 2005). Indeed, immune cells are selectively recruited into early neoplastic tissues, likely in response to hard-wired pathways utilized by all tissues to resist/repair damage caused by bacterial, viral, or other pathogenic assaults. When successful, "initiated" pre-neoplastic cells are purged by the immune system (Dunn et al., 2004). When the immune system fails, neoplastic cells are retained in "damaged" TMEs and provide a survival advantage resulting from abundant bioavailable mediators liberated as a function of tissue remodeling (Hanahan and Coussens, 2012). Ensuing neoplastic progression requires sustained presence of select immune subtypes that, combined with ongoing host-derived programs (angiogenesis, matrix, and tissue remodeling, etc.), contribute to cancer progression (Hanahan and Coussens, 2012) (Figure 2).

The classic view that immune cells merely facilitate tumor rejection has been supplanted by a more complex view of leukocytes having both tumor-promoting and tumor-inhibiting properties (Coussens et al., 2013). This is best explained by the existence of (at least) two types of inflammation with opposing effects on tumors: chronic inflammation, which promotes neoplastic cell survival, angiogenesis, tissue remodeling, and metastasis, and acute inflammation that triggers neoplastic cell destruction. While chronic inflammation is often linked with the presence of T$_H$2 responses, acute inflammation associated with cancer destruction is linked to T$_H$1 responses.

As neoplastic cells escape elimination, some become less immunogenic by downregulating MHC molecules; however, most if not all also activate intrinsic gene-expression programs that are inherently T-cell suppressive and myelo-stimulatory, e.g., T$_H$2 responses. Cytokines implicated in these scenarios include transforming growth factor β (TGFβ); IL-4, -13, -8, and -10; thymic stromal lymphopoietin (TSLP); and indoleamine 2,3-dioxygenase (IDO) (Coussens et al., 2013). This enables recruitment of FoxP3$^+$CD4$^+$ Treg cells, T$_H$2-CD4$^+$ T cells, T$_H$2-polarized macrophages and monocytes, and B regulatory cells (Bregs). In response to T$_H$2-mediated activation, myeloid cells commonly increase synthesis of angiogenic (e.g., VEGF), growth and/or survival (e.g., EGF, TNFα) factors that directly regulate epithelial cell proliferation, as well as tissue-remodeling enzymes (e.g., metallo-, cysteine, and serine proteases). These activities are remarkably pro-tumorigenic in that they nurture a TME favoring neoplastic cell survival and sustained proliferation (Balkwill et al., 2005). Simultaneous T$_H$2 activation of

macrophages and monocytes also increases expression of molecules, e.g., inducible nitric oxide synthase or Arginase 1, that directly and indirectly suppress CD8$^+$ T-cell proliferation and cytokines such as IL-10 that inhibit DC maturation and antigen cross-presentation to T cells (Ruffell et al., 2014). Thus, T$_H$2-type immune microenvironments are both tumor promoting and immune suppressive. Notably, in the colon, tumor-promoting immunity via IL-17 (T$_H$17)-mediated activation of myeloid and lymphoid cells has been reported (Wang et al., 2009; Wu et al., 2009).

Tumor-Promoting Activities of the Myeloid Compartment

Owing to their established role in wound healing, we investigated the ability of myeloid cells infiltrating early benign tissues to foster malignancy. In mice prone to squamous carcinogenesis, mast cells and macrophages activate pro-neoplastic angiogenic and tissue-remodeling programs (Coussens et al., 1999). In other studies of mice bearing mammary carcinomas, macrophages could regulate neoplastic cell dissemination and metastasis via EGF-mediated paracrine interactions with neoplastic epithelial cells (Lin et al., 2001). In human cancers, multiple studies have reported that the presence of macrophages in stroma correlates with aggressive disease and outcome (Komohara et al., 2014). Macrophages are recruited into tumors following activation of colony-stimulating factor 1 receptor (CSF1R) by either CSF1 or IL-34, two high-affinity ligands for CSF1R; the chemokine CCL2 may also facilitate macrophage recruitment (Qian et al., 2011). A CSF1-response gene-expression signature has been identified in 17%–25% of breast cancers associated with decreased estrogen receptor and progesterone receptor expression (Beck et al., 2009); serum concentrations of CSF-1 correlate positively with breast tumor size and predict poor survival (Aharinejad et al., 2013). In addition, in two independent breast cancer cohorts, intratumoral macrophage presence was correlated with potentially prognostic tumor features (high-grade, hormone receptor negativity; basal-like subtype; and increased risk of death) (Komohara et al., 2014). Macrophages therefore serve as promising targets for novel therapeutic interventions, particularly for patients with high-risk disease. Conversely, favorable prognosis has been associated with some tumor types exhibiting increased macrophage infiltration, e.g., non-small-cell lung cancer, prostate, colorectal, and gastric cancers (Komohara et al., 2014). Whether these distinctions reflect true differences in macrophage biology and function or arise due to discordant detection methodologies is unclear.

Neutrophils, on the other hand, are typically less abundant than macrophages in solid tumors, but their presence correlates with reduced survival in head and neck and breast cancers, and similar to macrophages, neutrophils develop polarized phenotypes that either favor or restrict tumor progression (Fridlender and Albelda, 2012). Recent studies identified granule products that suppress T-cell function (Sippel et al., 2011). Neutrophil expansion in mammary carcinomas of mice bearing mutant p53 alleles is driven by T-cell-derived IL-17; this results in systemic granulocyte colony-stimulating factor (G-CSF)-dependent expansion and polarization toward a T-cell-suppressive phenotype that facilitates metastatic dissemination and colonization (Coffelt et al., 2015). In contrast, neutrophils create a tumor-restrictive microenvironment in the lung that resists neoplastic progression and metastatic dissemination (Fridlender and Albelda, 2012).

Eosinophils, like other myeloid lineage cells, can exert cytotoxic immune-effector activities. Tumor-associated tissue eosinophilia (TATE) is associated with improved prognosis for a number of malignancies, including gastrointestinal, bladder, and prostate cancers; in contrast, TATE is associated with poor outcome in Hodgkin's lymphoma, cervical carcinoma, and oral squamous cell carcinoma (Davis and Rothenberg, 2014). Eosinophils have been associated with degranulation and release of cytotoxic proteins that mediate tumor rejection; recent results also reveal their role in normalizing the vasculature to improve CD8$^+$ T-cell trafficking associated with tumor regression (Carretero et al., 2015).

Monocytes, once in tissues, can differentiate into macrophages and DCs. Two circulating monocyte populations have been identified: classical inflammatory monocytes that are CCR2^{HIGH} and non-classical patrolling monocytes that are CX3CR1^{HIGH} (Geissmann et al., 2003). Recruitment of inflammatory monocytes into tissues is normally guided by the CCR2-CCL2 axis in response to parasitic or bacterial infections; in tumors, when CCR2^{HIGH} monocytes are recruited, they can promote neoplastic cell survival and extravasation through VEGF and CSF1 production (Qian et al., 2011). CCR2^{HIGH} monocytes promote survival of metastatic cells through a CCL3-dependent mechanism (Kitamura et al., 2015). CX3CR1^{HIGH} monocytes instead patrol capillaries in response to the CXC3R1-CX3CL1 axis; in these locales, they are positioned to scavenge particles and debris and thus are more likely to be found in wounds when inflammation is resolving. At sites of metastasis, CX3CR1^{HIGH} monocytes recruit NK cells that, in turn, kill metastatic cells, thereby providing a potent survival advantage (Hanna et al., 2015). In pancreatic adenocarcinomas, activation of the Ras oncogene leads to increased expression of GM-CSF and recruitment of immature monocytes that subsequently suppress CD8$^+$ T-cell proliferation to enhance tumor progression (Pylayeva-Gupta et al., 2012), analogous to other tumor systems (Gabrilovich et al., 2012).

Mast cells, present in all vascularized tissues, respond to diverse stimuli by either secreting or releasing (via degranulation) biologically active compounds, e.g., proteolytic enzymes, heparin, histamine, prostaglandins, cytokines, and chemokines. Mast cells are key for maintaining tissue homeostasis and are best known for their effector functions following IgE-stimulated allergic responses and anaphylaxis (Metz et al., 2007). Mast cells have been implicated in the vascularization of a multitude of solid human tumor types, likely owing to their proteolytic products and high VEGF expression following activation (Coussens et al., 1999; Marichal et al., 2013) following CCL2-mediated recruitment where their bioactive mediators promote neoplastic progression.

Tissue Specificity of Myeloid Programming

While it is conceptually unclear how some myeloid cells adopt a T$_H$2 or protumorigenic state to support neoplastic progression, some clues have emerged in recent genetic studies. Several groups revealed that lymphocytes drive initial myeloid cell programming to foster chronic inflammation in a tissue-specific manner. For example, during mammary branching morphogenesis and ductal carcinogenesis, cytokines derived from

T_H2-CD4$^+$ T cells, e.g., IL-4 and -13, activate macrophages and monocytes infiltrating mammary tissue (DeNardo et al., 2009; Plaks et al., 2015). In neoplastic scenarios, signaling downstream of IL-4 receptors on monocytes and macrophages triggers protumorigenic T_H2 gene-expression programs that activate tissue-remodeling cascades via expression and activation of cathepsin proteases and immune-suppressive programs via upregulation of IL-10 and immune-checkpoint molecules (DeNardo et al., 2009; Gocheva et al., 2010; Mitchem et al., 2013; Ruffell et al., 2014). Mast cells and macrophages (as well as other myeloid cell types) are T_H2 programmed in early squamous and pancreatic carcinomas by a diversity of pathways, which also include activation of immunoglobulin receptors (FcγRs) by immune complexes (ICs) (Affara et al., 2014; Andreu et al., 2010). ICs are composed of antigen-specific antibodies and complement proteins that variably activate FcR and complement receptors depending on composition of IC and status of the myeloid cell being activated (Karsten and Köhl, 2012). While these humoral immune-regulated paracrine programs were known to shape outcomes in chronic inflammatory diseases, recognition of their significance in solid tumors was paradigm shifting and highlighted the significance of hard-wired tissue-specific programs shaping host response to disease. These data illustrate the diversity of pathways utilized by innate immune cells to propel cancer by directly enhancing tissue-based programs favoring survival of neoplastic cells, in concert with direct and indirect activation of programs to extinguish cytotoxic immune responses aiding immune escape (Figure 2).

T_H2-Based Targets for Anti-cancer Therapy

The collective evidence not only supports a protumorigenic role for chronic inflammation in cancer, but also indicates that inflammation is malleable, akin to the healing of acute wounds during which immune cells toggle between T_H1 and T_H2 states. Thus, the hypothesis that T_H2-driven myeloid cells could be re-programmed, or at least neutralized, to reduce the presence or immunosuppressive status of macrophages, trigger anti-tumor immunity, and/or suppress tumor growth has been tested in several tissue-specific cancer models. We and others have evaluated CSF1-neutralizing monoclonal antibodies (αCSF1 mAB) and small-molecule CSF1R inhibitors for their ability to suppress macrophage survival and/or presence in tumors, in combination with chemotherapy (CTX) or radiation therapy (RT) (DeNardo et al., 2011; Ruffell et al., 2014; Shiao et al., 2015). These studies reveal increased chemo- and radiation sensitivity associated with anti-tumor immune responses directed by CD8$^+$ T-cell infiltration of tumors, culminating in reduced primary tumor growth and metastasis with increased survival. Other preclinical studies revealed that CSF1/CSF1R blockade, as monotherapy or combined with CTX/RT, improved outcomes for glioma, prostate, and pancreatic adenocarcinoma, as well as melanoma, whereas CSF1R blockade improved antitumor efficacy of immune checkpoint blockade and adoptive T-cell therapy (reviewed in Ruffell and Coussens, 2015). Importantly, administration of RG7155, a CSF1R-blocking mAb, in patients with diffuse-type giant cell tumors, reduced CSF1R$^+$CD163$^+$ macrophage levels; this translated into objective clinical responses (Ries et al., 2014). Treatment of tenosynovial giant-cell tumors with a small-molecule

inhibitor of CSF1R kinase increased progression-free survival and improved outcomes as a monotherapy (Tap et al., 2015). The macrophage presence in tumors has also been therapeutically manipulated by targeting the macrophage signaling protein acting through its transmembrane receptor kinase RON, wherein activation of RON in macrophages favors conversion of micrometastatic lesions to overt metastases by suppressing antitumor immune responses. Functional RON blockade in preclinical models potentiates tumor-specific CD8$^+$ T-cell responses, indicating that RON inhibitors may also improve outcomes for cancer patients (Eyob et al., 2013).

Bruton's tyrosine kinase (BTK) is an attractive target, as BTK is activated downstream of the B-cell receptor and FcγR and PI3Kγ in some myeloid subsets (Smith et al., 2001). In vitro, neoplastic cell challenge via co-culture with splenic cells from B-cell-deficient versus B-cell-proficient mice revealed that IFNγ release from CD8$^+$ and NK cells is increased when B cells were absent, whereas presence of B cells or B-cell-derived IL-10 was associated with reduced IFNγ (Inoue et al., 2006). Though these in vitro studies indicate that B cells can direct T-cell responses, the role of myeloid cells as mediators of these responses is now clear and indicates that therapies targeting common pathways in B cells and/or myeloid cells, such as SYK, BTK, PI3Kγ, may be efficacious in solid tumors, analogous to efficacy observed for BTK and PI3Kδ inhibitors in B-cell malignancies (Hendriks et al., 2014). This concept was recently validated preclinically, whereby BTK inhibition enhanced survival of mice bearing pancreatic adenocarcinoma (Gunderson et al., 2016; Massó-Vallés et al., 2015), neuroendocrine cancers (Soucek et al., 2011), and other subcutaneous tumors (Sagiv-Barfi et al., 2015) in which a common feature was reduced inflammation and inflammatory desmoplasia with evidence of macrophage repolarization.

If these preclinical findings are any indication, immune therapies targeting macrophages and/or other protumorigenic immune cells could alter the human tumor immune microenvironment in a way that fosters cytotoxic properties of CD8$^+$ T cells. As immune-checkpoint inhibitors of pathways regulating T-cell activity are proving efficacious for subsets of cancer patients, we predict that combining these two immune-based approaches represents a compelling clinical opportunity. However, it is likely that not all tumors will respond; thus, identifying predictive biomarkers and correlates of therapeutic response is a top priority. Based on preclinical data evaluating macrophage antagonists and checkpoint inhibitors, we predict that biomarkers of response will also be reflected by changes in peripheral blood. Such biomarkers will form the basis for simple, non-invasive diagnostic and/or prognostic screens aiding early detection in susceptible populations (Figure 4).

In preclinical models, regardless of tumor or approach, T_H1 immunity emerges when dominant T_H2-driver pathways are attenuated; when concomitant with cytotoxic therapy, tumor growth stalls or regresses by CD8$^+$ T-cell-dependent mechanisms. These findings highlight the importance of neutralizing pro-tumor inflammation as a therapeutic strategy and indicate that tumor-infiltrating CTLs can be mobilized in tumors with low mutational burdens. These data also highlight the clinical need for biomarkers that identity tissue-specific programs driving T_H2 immune responses; such data is needed to inform
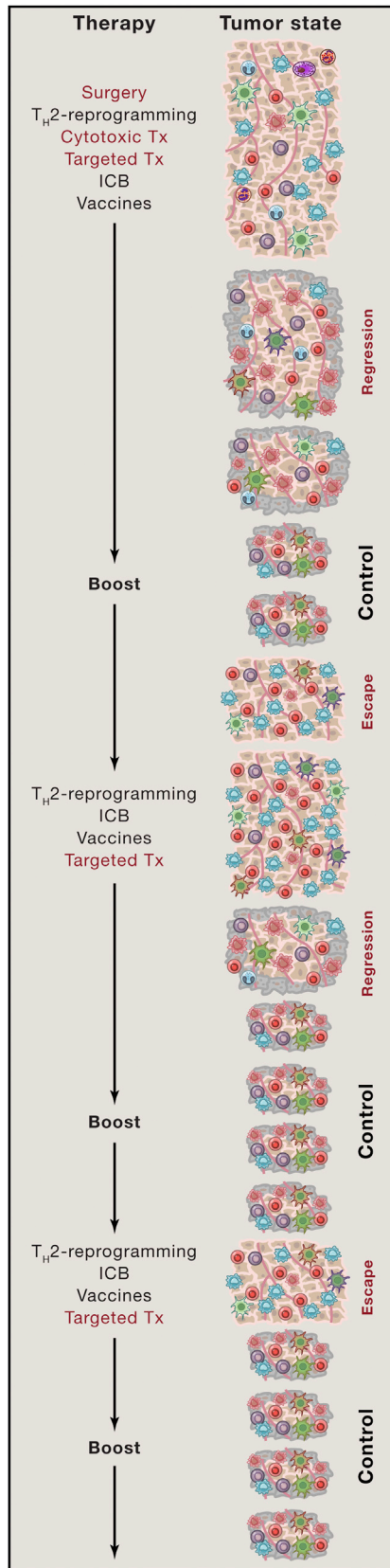
Figure 4. Multi-Modal Biomarker-Based Approach for Optimal Immune-Mediated Tumor Control

Cancer medicine is evolving. Going forward, individuals with cancer will be evaluated for biomarkers enabling stratification to determine most optimal combinations for therapy based on tumor-based and systemic biomarkers. Eventually, all patients with cancer will be treated with checkpoint inhibitors, either directly or after interventions targeting inflammation (for example, with T$_H$2-blockade therapies, radiation therapy, or epigenetic modulation), or vaccination via DCs to boost T-cell repertoires, or adoptive T cell transfer. Based on the known tissue-embedded programs empowered to control autoimmunity, it is reasonable to anticipate that a majority of patients will develop acquired resistance followed by immune escape; this will lead to the next cycle of treatments incorporating multi-modal biomarkers (e.g., based on microbiome phenotype, circulating cell-free DNA [cfDNA], circulating cytokine levels) and perhaps NK cells recognizing loss of MHC class I by neoplastic cells, thus rendering them invisible to T cells. Cytotoxic treatments such as with NK cells or standard cytotoxic therapy (CTX or RT) or oncolytic viruses will release neo-antigens that can be used for generation of the next round of effector T cells. Whole-exome sequencing (WES) of tumor samples as well as cfDNA will yield information on mutational load that can, in turn, be used as one class of neo-antigens for vaccination and priming of new T-cell repertoires. T-cell receptors (TCR) can be assessed using genomic approaches enabling sequencing of TCRβ chains to assess repertoire diversity. Given the importance of T-cell specificity for relevant antigens, strategies enabling paired sequencing of α and β TCR chains will be invaluable, as will high-throughput tetramer analysis. In addition, RNA-seq and epigenetic analysis of tumors and their infiltrates will enable assessment of the type and flavor of inflammation. Future studies will incorporate metabolomics to this biomarker portfolio.

precision medicine strategies employing T$_H$2 blockade, in concert with other immune, targeted, or cytotoxic approaches (Figure 4).

Immune-Targeted Therapies Focused on T Cells
Basic Principles

Cancer immunotherapy historically relied on two principal mechanisms of action: (1) "passive" immunotherapy via provision of anti-tumor antibodies, e.g., Trastuzumab (αHER2 mAB) or Rituximab (αCD20 mAB), or adoptive transfer of cytotoxic T and NK cells; and (2) "active" immunotherapy that mobilizes the patient's immune cells via checkpoint blockade, i.e., administration of antibodies directed against immune-regulatory checkpoint molecules expressed on T cells or via vaccines that expand antigen-specific T cells. In all circumstances, T cells are the drug; we are learning that T cells have the ability to clinically control some cancers (Postow et al., 2015). T cells can be targeted in three major ways: (1) by being liberated by checkpoint inhibitors; (2) through adoptive transfer when T cells are missing, as validated by the clinical success of genetically engineered T-cell therapies; and (3) through induction in vivo by vaccination or endogenous mechanisms subsequent to other anti-cancer therapies (e.g., CTX, targeted therapies, or anticancer antibodies) (Palucka and Banchereau, 2013).

Immune Checkpoint Blockade

Immune checkpoint blockade (ICB) can unleash the power of naturally occurring T cells by eliminating negative signals that block T-cell function (Pardoll, 2012). ICB has produced durable clinical responses and improved survival across a variety of cancers (Topalian et al., 2015). CTL expansion and function are carefully regulated by cytotoxic-T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and other molecules so as to maintain a delicate balance between the resolution of infection, the elimination of infected cells, the protection of tissue homeostasis, and the prevention of autoimmune

attack. CTLA-4 is a cell-surface receptor expressed by activated T cells with homology to the T-cell costimulatory molecule CD28. Although CD28 and CTLA-4 are both ligands for B7-1 (CD80) and B7-2 (CD86), they serve opposing roles in regulating T-cell activation. CD28 provides costimulatory signals required for T-cell activation, whereas CTLA-4 negatively modulates T-cell responses by raising the activation threshold for T-cell priming; thus, CTLA-4 is likely most important during priming. PD-1 binds programmed death ligand 1 (PD-L1; a.k.a., B7-H1 or CD274) expressed by neoplastic cells, various immune cells, mesenchymal support cells, and vascular cells; this interaction negatively regulates T-cell activation when engaged with an APC and/or effector function when engaged with other PD-L1-positive cells. Indeed, binding of PD-L1 to its receptors suppresses T-cell migration and proliferation and restricts cancer cell killing (Topalian et al., 2015); thus, PD-1 is important in regulating effector functions after CD8$^+$ T cells are activated. PD-1 and CTLA-4 regulate distinct phases of T-cell differentiation and function, and their inhibition might need to be optimally phased for maximum efficacy. This concept needs to be incorporated in the next generation of clinical trials of combination therapy regimens, especially when combined with vaccines or agents that reprogram myeloid cells to foster a T$_H$1-type activation state.

Indeed, combination therapies targeting the two checkpoints, i.e., CTLA-4 and PD-1, further increase progression-free survival in patients with metastatic melanoma (Larkin et al., 2015); however, in other cancers, these responses are present in fewer patients. Resolving the natural and acquired resistance to checkpoint inhibition therapy represents the next frontier in basic research and clinical development (Figure 4). As the effector arm of checkpoint inhibition, T cells could underpin the major resistance mechanisms for checkpoint blockade. Thus, non-responding patients might actually lack naturally occurring T cells with specificity against neoplasias, and/or their T cells could be held hostage and rendered dysfunctional in TMEs via pathways other than checkpoints, such as immune-suppressive microenvironments directed by T$_H$2-activated myeloid, Treg, or Breg cells (Coussens et al., 2013). Links between treatment resistance and T-cell shortage are supported by recent findings that tumor-specific mutations generate neo-antigens that, in turn, may drive anti-tumor responses. Indeed, whole-exome sequencing of malignant melanomas from patients treated with CTLA-4 blockers demonstrated an association between mutational load and degree of clinical benefit (Snyder et al., 2015); however, in other melanoma cohorts, recurrent neo-antigen peptide sequences were not found to predict responder populations (Van Allen et al., 2015). In non-small-cell lung cancers treated with αPD-1 mAb, higher mutation burden in tumors was associated with durable clinical benefit and progression-free survival (Rizvi et al., 2015). Neo-antigens arising as products of somatic mutations are not presented in the thymus; thus, they can be recognized by the immune system as foreign antigens, similar to viral antigens or organ transplants, because the T cells have not been eliminated or tolerized. These concepts were put forward early (Srivastava, 2000) but validated only recently (Schumacher and Schreiber, 2015), thanks to the availability of massively parallel sequencing.

Cancer Vaccines

Patients may fail or resist checkpoint therapy owing to a lack of pre-existing T cell infiltrates. Therefore, vaccination and adoptive transfer strategies to first induce and expand the breadth of endogenous T-cell responses could prove useful. Vaccines are composed of antigens and adjuvants. Responses to vaccination and adjuvants involve DCs that capture and present vaccine antigens, thereby facilitating differentiation of lymphocytes and subsequent immunity (Figure 3). DCs also integrate the adjuvant signals and determine the quality of induced immune responses.

Several phase III clinical trials testing various cancer vaccine platforms, including DC-based and viral-vector-based vaccines, are ongoing. These exogenous vaccine platforms will need to be accompanied by high-throughput genomics to incorporate personalized cancer-specific mutations and candidate peptide antigens. Indeed, proof-of-concept trials in patients with advanced melanoma demonstrated that naturally occurring neo-antigen-specific immunity was enhanced by vaccination with DCs loaded with patient-specific peptides (Carreno et al., 2015). Another concept is endogenous vaccination based on antigen released upon standard CTX/RT or oncolytic viruses (i.e., viruses that preferentially infect and kill cancer cells); this, however, requires effective antigen presentation to generate therapeutic T-cell immunity (Palucka and Banchereau, 2013). DCs are skewed by tumors toward pro-tumor immunity; thus, reprogramming to foster T$_H$1-skewed mature functionality in vivo is critical for success of endogenous vaccination. Our understanding of cancer genomics, the biology of antigen presentation, and T-cell biology has advanced impressively and continues to increase; this knowledge will feed into the development of the next-generation cancer vaccines that, combined with checkpoint inhibitors, hold promise for improving outcomes for cancer patients (Figure 4).

DC vaccines can be exploited as anti-cancer therapeutics through various strategies, including: non-targeted peptide/protein and nucleic-acid-based vaccines captured by DCs in vivo; vaccines composed of antigens directly coupled to anti-DC-antibodies; or vaccines composed of ex-vivo-generated DCs loaded with tumor antigens (Palucka and Banchereau, 2013). DCs are also engaged in response to complex vaccine preparations such as GVAX, a tumor-cell-based vaccine in which neoplastic cells genetically modified to express GM-CSF attract and activate DCs (Le et al., 2010). Other vaccine platforms are based on recombinant-attenuated *Listeria monocytogenes* (*Lm*), an intracellular bacterium that targets DCs in vivo and utilizes MHCI and II antigen-processing pathways (Le et al., 2012), as well as intratumoral delivery of oncolytic viruses; these can be modified to express GM-CSF to attract DCs and lymphocytes at the lysed tumor site (Russell et al., 2012). Finally, pioneering studies from Ralph Steinman and Michel Nussenzweig demonstrated the principle of targeting antigens to DCs in vivo through coupling of antigens to antibodies specific to DC surface receptors such as DEC205 or DCIR (Bonifaz et al., 2002; Soares et al., 2007). Importantly, in the absence of adjuvants, targeting antigens to DEC205$^+$ DCs in vivo induces antigen-specific tolerance (Hawiger et al., 2001). Administration of these complex vaccines with DC activators such as TLR3, TLR7-8, or CD40 agonists enables maturation of DCs and

consequent establishment of immunity rather than tolerance (Bonifaz et al., 2002; Soares et al., 2007). It remains to be seen which vaccine platform will be most effective at priming and boosting T cells in patients; this clearly represents the next frontier in research.

T-Cell-Dependent Nature of Cytotoxic and Targeted Therapy

Cancer medicine evolved largely based on the principle that rapidly proliferating malignant cells can be eradicated by cytotoxic regimens (CTX or RT) or by targeted drugs attacking attributes of mutationally corrupted cells. As discussed above, the recent advent and remarkable efficacy of immune-checkpoint inhibitors revealed the clinical potential of harnessing endogenous mechanisms of anti-tumor immunity in tumors harboring significant mutational burdens. Upon reflection, however, it is appreciated that conventional cytotoxic approaches modulate the composition and functional bioactivity of intratumoral leukocytes, in addition to effects on neoplastic cells (Galluzzi et al., 2015). Furthermore, in some scenarios, the efficacy of neoadjuvant CTX correlates with increased presence of intratumoral immune-effector T cells (Galluzzi et al., 2015). These correlations are not limited to cytotoxic regimens—the tyrosine kinase inhibitor imatinib also leads to increased presence of CTLs and NK cells in gastrointestinal tumors in a manner that correlates with disease outcome (Kroemer et al., 2013), while efficacy against chronic myelogenous leukemia can be reversed by co-administration of type I IFN (Galluzzi et al., 2015). A recent study in breast cancer also revealed that efficacy of transtuzumab emtansine is linked to elicitation of anti-tumor immune responses (Müller et al., 2015).

Malignant cells can emit danger signals, albeit distinct from those of normal tissue, that are sensed by immune cells and thus are antigenic. Increased antigenicity is linked to either mutational burden, where peptides from mutant proteins are presented by MHC molecules (Gubin et al., 2014), or to ectopic expression of cancer testis or oncofetal antigens typically only expressed during embryonic or fetal development (Whitehurst, 2014). The increased adjuvanticity of neoplastic cells is linked to metabolic stress caused by their sustained proliferation and to their ability to adapt and survive in hypoxic TMEs (Krysko et al., 2012). Furthermore, preclinical data have emerged supporting the proposition that tumors treated with conventional CTX engage antigenic and adjuvant immune-mediated mechanisms. In murine tumor models, the anti-neoplastic effects of anthracyclines are significantly reduced when either $\gamma\delta$ or CD8$^+$ T cells are depleted, but not when B or NK cells are absent, DC infiltration is blocked or corrupted, immune-stimulatory type I IFNs or IL-17 are lacking, or DAMP-mediated recruitment and activation of effector cells is thwarted (Kroemer et al., 2013). Cyclophosphamide, oxaliplatin, and bortezomib similarly rely on immune-mediated mechanisms for their efficacy; these commonly used cytotoxics elicit effector cell activity via plasma membrane exposure of calreticulin and release of the chromatin-binding protein high-mobility group box 1 (HMGB1). This, in turn, fosters DC maturation and TLR4 and RAGE activation (Apetoh et al., 2007), thus increasing adjuvanticity of malignant cells.

Taxanes, broadly used microtubule inhibitors, and vinca alkaloids promote polyploidization due to mitotic interference, thus leading to endoplasmic reticulum stress responses favoring calreticulin exposure and immune-mediated elimination (Senovilla et al., 2012). Clinically, docataxel, vinorelbine, and cisplatin all lead to increased abundance of circulating CTLs and decreased presence of Treg cells and immature myeloid cells harboring T-cell-suppressive activity; this latter effect is also shared by gemcitabine, a common CTX for pancreatic adenocarcinomas, and 5-fluoruracil. Interestingly, paclitaxel is also a TLR4 ligand and thus enhances T-cell priming by DCs (Pfannenstiel et al., 2010).

Cyclophosphamide also provokes relocalization of intestinal gram-positive bacteria to secondary lymphoid organs, resulting in generation of T$_H$17 cells secreting IL-17 and IFNγ that promote anti-tumor immune responses (Viaud et al., 2013). In murine tumor models, therapies targeting T$_H$2-based programs (e.g., CSF1R or RON antagonists, BTK or SYK inhibitors, B-cell depletion, αIL-4 or αIL-13 mAbs) enhance efficacy of either CTX or RT by T-cell-dependent mechanisms (Ruffell and Coussens, 2015). Perhaps the most compelling evidence is that provided by recent data revealing that immune-checkpoint blockade, when combined with CTX, improves overall survival in several cancer types beyond CTX alone (Topalian et al., 2015). The ability of these agents to activate adaptive stress-response pathways and send danger signals operative as immunologic adjuvants inherently increases the antigenicity of tumors even when mutational burden is low. These untoward effects can be capitalized upon to improve outcomes for individuals with cancer.

A Role for the Microbiome in Regulating Systemic Cancer Risk and Response to Therapy

If the precision medicine equation wasn't sufficiently complicated by neoplastic cell genomics, epigenomics, host immune responses, and the TME, mounting evidence points to an additional consideration when attempting to stratify patients and predict therapy response: the host microbiome. The context and composition of common microorganisms living in the gut not only shapes local immune responses, but also regulates systemic immunity and thus impacts the risk of and progression to malignancy and the response to anti-cancer therapies. Intra-abdominal infections and use of antibiotics has long been associated with increased incidence of colorectal cancer (Wang et al., 2014). In mouse models, attenuating or selectively altering the composition of gut microorganisms influences both the incidence and progression of cancer (Zitvogel et al., 2015). Intestinal microorganisms not only impact local risk of tumorigenesis, but also influence neoplastic progression distally by altering inflammatory and metabolic circuitry. These experimental results correlate with epidemiologic data revealing increased incidence of breast cancer in women with significant history of antibiotic use (Zitvogel et al., 2015).

Gut microbiota composition is dramatically impacted by common anti-neoplastic drugs, including RT, allogeneic stem-cell transplantation, and select CTXs, notably 5-fluorouracil and irinotecan (Zitvogel et al., 2015). Along these lines, the gut microbiota affects the amenability of some tumor types to therapy by impacting regulatory aspects of the immune response.

Examples include translocation of gut microbiota across the intestinal epithelium in response to lympho-depleting irradiation in which DCs are inadvertently activated, leading to altered serum cytokines and improved responses to adoptively transferred CTLs; these beneficial effects are abated by antibiotics (Paulos et al., 2007). Similarly, cyclophosphamide alters composition of gut microbiota, resulting in translocation of gram-positive bacteria into secondary lymphoid organs wherein pathogenic T_H17 and memory T_H1 cells are activated; tumors grown in germ-free mice, or antibiotics tropic for gram-positive bacteria exhibit reduced T_H17 responses and tumor resistance to cyclophosphamide (Viaud et al., 2013). Antibiotic eradication of gram-positive bacteria also impairs the efficacy of CpG-oligo-nucleotide immunotherapy and platinum CTX by altering myeloid cells within the TME (Iida et al., 2013). *Bifidobacterium* occupancy supports anti-tumor immunity against melanoma and improves the efficacy of αPD-L1 and αCTLA-4 mAb therapy by altering DC activity, leading to improved antigen-specific CD8^{+} T cell function—these effects were reduced by ampicillin, colistin, or streptomycin but were enhanced by vancomycin due to preferential enhancement of *Bacteroidales* colonization (Vétizou et al., 2015). These data underscore the impact of gut commensals on therapeutic responsiveness.

Could selectively manipulating the gut microbiota impact risk of developing cancer, limit incidence of select tumor types, and/or improve activity of some anti-cancer therapies? Zitvogel and colleagues have proposed four distinct approaches for manipulating the gut microbiota to boost cancer therapy: (1) preferential use of antibiotics selective for untoward bacterial species; (2) increased use of probiotics; (3) increased use of prebiotics to stimulate healthy gut colonization; and (4) use of postbiotics, nonviable products of microbiota that exert biological activities in hosts (Zitvogel et al., 2015). Prospective stool analysis and monitoring in cancer patients receiving therapy will surely reveal biomarkers that, if harnessed, could improve patient stratification and/or support new microbiota-based strategies for boosting therapeutic responsiveness, e.g., fecal transplant of beneficial species.

Multi-Modal Tumor and Systemic Biomarkers for Stratification and Resistance Monitoring

A major clinical goal is to understand the multi-modal tissue-based and systemic pathways regulating therapy responses so as to minimize resistance and maximize efficacy of cancer medicine (Figure 4). Whether therapies target tumor-intrinsic pathways, host pathways, or commensal microbiota, it is clear that understanding non-genomic mechanisms of resistance from an integrated standpoint is critical.

Understanding which immune cell types are present in and around a tumor currently provides invaluable retrospective information regarding tumor ecology and/or tumor response to therapy. However, we must improve our ability to integrate information on not only the complexity of leukocytes in tumors, but also their geography in tumor nests and stroma. Immune cells are scattered in tumor core and within tumor stroma, in invasive margins, and in organized lymphoid structures often distant from neoplastic cells. Investigating the mechanisms governing formation of tertiary lymphoid structures (TLSs) found in numerous cancers represents a new frontier for biomedical research. Such topology has been reported by Galon and colleagues to be clinically meaningful in colorectal cancer, where a statistically significant correlation between immune cell density and patient outcome was revealed (Galon et al., 2006). Moreover, development of TLS in individuals with pancreatic adenocarcinoma treated with vaccines correlated with improved clinical outcomes (Le et al., 2015). Furthermore, compared with single-region analysis, combined analysis of the tumor core and invasive margins improved the accuracy of survival prediction in different patient groups (Galon et al., 2006). These early results form the basis for immune stratification of patients, or the so-called Immunoscore, and its coordinated assessment in the clinic (Ascierto et al., 2013). An international consortium has been initiated to validate and promote the Immunoscore in routine clinical settings (Ascierto et al., 2013); results of this international effort may lead to implementation of the Immunoscore as a new classification metric, designated TNM-I (TNM-Immune).

Will the Immunoscore provide enough additional information to prospectively predict response to therapy? Likely not. We predict that integrating the Immunoscore with additional metrics will be critical for guiding patient stratification and phasing of combinatorial therapies. Such metrics will include genomic and exomic features of neoplastic cells (through sequencing of neoplastic cells themselves or cfDNA, tracking the expansion of tumor-specific CD4^{+} and CD8^{+} T cells, monitoring serum cytokine fluxes, and evaluating the composition and health of commensal bacteria (Figure 4). Serum cytokines have long been enigmatic due to their labile nature and the detection limitations of conventional methodologies. That said, serum biomarker signatures are now able to discern asymptomatic early stage pancreatic cancer from healthy controls with 96% accuracy (Ghatnekar et al., 2013) and can be used to monitor the pharmacodynamics of CSF1R-targeted therapies (Butowski et al., 2015). Moreover, transcriptional profiling of blood monocytes in renal cell carcinoma identifies biomarkers correlating with tumor staging (Chittezhath et al., 2014), and mRNA sequencing of tumor-educated blood platelets distinguishes cancer patients from healthy individuals with 96% accuracy (Best et al., 2015). Thus, multi-modal functional diagnostic strategies integrating the tumor, host, and commensals will likely forge the advent of next-generation precision bioinformatics to match patients with appropriately combined and phased anti-cancer therapies.

Oncoimmunology Treatment Paradigm

Future immunotherapies will be based on cycles of interventions designed to boost and modulate anti-cancer immunity (Figure 4). Indeed, as we rediscover and refine the fundamental principles of tumor immunology, it is increasingly clear that curing cancer might not be a realistic goal. Rather, aiming for a continuum of treatment cycles designed and based on mechanistic in vivo studies and in-depth analysis of each patient's tumor will be necessary for optimizing outcomes. Clinical trials with checkpoint inhibitors teach us that in situ immune infiltration is critical for tumor regression; however, not all immune infiltrates are equal, and as discussed throughout this article, the quality of immune response is a critical factor for therapeutic success. This,

in turn, is determined by underlying inflammation, which we assert must become a staging parameter, along with classical pathology-based schemas and the Immunoscore. It will also need to be established to what extent inflammation, which clearly plays a role in epithelial tumors, impacts other tumor types, e.g., melanoma or sarcomas. Additional parameters pertain to the specificity of infiltrating T cells against cancer antigens, as again, the infiltrate with passenger T cells might not be therapeutically useful and should be tested. Eventually all patients will be treated with checkpoint inhibitors, either directly or after interventions targeting inflammation, by vaccination to boost T-cell repertoires or by adoptive T-cell transfer. The majority of patients will subsequently develop acquired resistance followed by immune escape; this will lead to the next cycle of treatments incorporating multi-modal biomarkers (e.g., based on microbiome phenotype, cfDNA, circulating cytokine levels) and perhaps NK cells recognizing loss of MHC class I by neoplastic cells, thus rendering them invisible to T cells. Cytotoxic treatments, such as with NK cells, standard CTX/RT, or oncolytic viruses, will release neo-antigens that can be used to generate the next round of effector T cells. To this latter point, we must fully understand the rules of T-cell priming in vivo in humans, identify the most effective ways to utilize DCs for priming, and develop strategies for mobilizing the naive T-cell repertoire from the thymus in adults (Sportès et al., 2008). In later rounds of therapy, the scope of neo-antigens will likely be broadened as, in addition to somatic mutations, neo-antigens can be generated via epigenetic and post-translational regulation. Last but not least, the role of Tregs, so well established in murine cancer, will need to be redefined in humans. Resolving all of these challenges will surely keep us busy for a long while.

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